MALARIAL PARASITOLOGY.*

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INTRODUCTION.

Although the chief interest in the investigation of malaria centres at present in the study of experimental inoculations, of the natural mode of infection, and of the extracorporeal forms of the parasite, much yet remains to be learned about the morphology of the organism in its several varieties. The minute study of the morphology of the parasite has furnished far more cogent evidence of the existence of several species of the plasmodium of human malarial fever than has that of the clinical manifestations of the disease. Yet even the tantamount question of the unity or plurality of species is still far from satisfactory solution. Moreover, many details in the biology of the plasmodium of malaria, including the development of the crescentic bodies, whose significance has been partly determined, still remain

* Received for publication December 8, 1899.

obscure and require further observations on the occurrence and behavior of the parasite in the blood.

My observations on a considerable number of cases of Cuban malarial fever, seen at Montauk during the summer of 1898, although pursued primarily for the purpose of immediate diagnosis, bear on the topics mentioned. The conclusions reached at that time and more especially the deductions drawn from a more leisurely review of the material then obtained it is proposed to consider in the present article.

I. TECHNICS.

Experience with the technics of blood examinations in malaria has led me to restrict the use of fresh blood to the study of various vital phenomena in the parasite, such as amœboid movement, vibratory motion of pigment, and exflagellation. When parasites are scanty their discovery is so much more certain and rapid in stained dry specimens that a negative result with fresh blood invariably requires verification by search through a dry specimen, stained preferably by Nocht's method. Moreover, exclusive reliance upon fresh blood not only leads constantly to errors in diagnosis by beginners, but also has been the cause of many erroneous conceptions held in the past by experts.

The study of flagellate bodies may be conducted in fresh specimens prepared in the ordinary way, but placed by preference on a warm stage. The addition of a little water may facilitate the escape of the parasite from the cell and the formation of flagella. The successful action of water has been obtained by several expedients. Marshall added about an equal quantity of water to a small drop of blood containing many crescents and saw the almost immediate change of crescents to spheroidal bodies, followed by flagellation. Manson[1] recommends that the blood under the cover-glass be kept moist by exposure to steam exhaled from a hot, moist sponge. After a few minutes the cover may be carefully removed, the specimen dried, and the flagellate body stained.

Bignami studied in detail the flagellate bodies in specimens rather thickly spread, and prevented from drying in a warm moist chamber, while exflagellation occurred, with subsequent drying and staining. I find that a moist chamber may be conveniently secured in a Petri dish with tightly fitting vaselined cover. Wet blotting paper placed in the dish furnishes the necessary moisture. Specimens spread on slides or

[1] *Lancet*, 1896, ii, p. 1715.

covers may be kept moist for 10 to 20 minutes in such dishes, and flagellation proceeds with moderate rapidity.

Staining Methods.

1. *Eosin and Methylene Blue.*—For all ordinary purposes staining by eosin and methylene blue may be generally recommended, and was largely employed in the present cases. The solutions required are: (*a*) a saturated alcoholic solution of alcoholic eosin diluted with an equal quantity of 95 per cent alcohol, and (*b*) a saturated watery solution of Ehrlich's rectified methylene blue at least one week old.

A light staining by eosin, such as is given by the diluted solution of eosin, is essential for the clear demonstration of the parasite by methylene blue, and in specimens containing only the small signet-ring forms, heavy staining by eosin may almost entirely prevent the subsequent action of methylene blue, and these minute parasites may thus be overlooked.

2. Methylene blue fails to stain the young ring forms, especially those of the tertian type, as clearly as is desirable, and more powerful basic staining fluids may well be employed for this purpose. Nocht's method may be recommended over any other, as it facilitates the identification of the small ring by means of a large densely stained nucleus, but when this method cannot be employed, one may resort to the method modified by Futcher and Lazear from the suggestions of Benario and Marchoux, as follows: Fix the specimens five minutes in 95 per cent alcohol, 100 cc., to which has been added 1 cc. of formalin. Stain one to three minutes in the following mixture: sat. alc. sol. thionin, 20 cc., 20 per cent carbolic acid, 100 cc. The fixing solution must be used fresh, and the staining fluid must be at least one week old. The rings are then densely stained and the specimens do not fade.

3. The sharpest demonstration of minute ring-shaped parasites was obtained in the present cases by staining one hour in diluted Gage's hæmatoxylin, before treatment by eosin and methylene blue. Hæmatoxylin stains the nucleus of the ring and makes the body of the parasite blacker after methylene blue. Such specimens are specially suitable for photography.

4. *The Nocht-Romanowsky Method.*—In 1890 Romanowsky published a method of staining the malarial parasite, which apparently demonstrated certain nuclear structures very imperfectly shown by other methods. The original description of this method directed that the specimens, fixed in equal parts of alcohol and ether, or by heat at

110° C., be placed for two to three hours in a fresh mixture made by adding concentrated (1 per cent) solution of methylene blue, 1 part, to 1 per cent watery solution of aqueous eosin, 2 parts. The red corpuscles were stained light pink, and the body of the parasite blue, while the chromatin particles of the nucleus appeared deep red.

The sketches accompanying Romanowsky's article were absolutely convincing that the new method was a most valuable addition to existing technical procedures, but it was soon found that the results obtainable from this method were extremely uncertain. We now know that only those who happened to secure particular specimens of methylene blue obtained successful results with Romanowsky's stain, and that the later highly purified products of the anilin-dye factories seldom contained the effective agent which united with the chromatin granules. Romanowsky believed that by the mixture of methylene blue and eosin a third compound was formed which stained the chromatin. Attention was not fully drawn to this probable explanation of the difficulty until Ziemann published the results of his experience with various specimens of methylene blue. After numerous attempts to stain by Romanowsky's method, following the exact directions, he sums up his results in the few words: "glückte kein Präparat." After a long series of experiments he found that a successful result depended upon the variety of methylene blue, the age of the solution, the quantity of eosin used, and the length of exposure. He secured most uniform results from the use of 1 per cent aqueous solution of methylene blue (med. puriss., Höchst) or Koch's methylene blue (Grübler), or Ehrlich's rectified methylene blue (Grübler). He found no difference in the effects of various specimens of eosin, but used principally a 1 per cent aqueous solution of watery eosin (Höchst). The proportions of these solutions required varied from 1 to 7 parts of eosin added to 1 part of methylene blue, and the time of staining from 15 to 40 minutes. The proportions of the dyes and the time of staining had to be determined for each specimen of dye. The older the solution of methylene blue, the less eosin was required in the mixture.

Gautier, who was very successful with Romanowsky's method, had the best results by using specimens of methylene blue marked C and BGN, and of eosin marked A, of the Baden Anilin factory. He found considerable difference in the results obtained with different specimens of eosin. Many other observers had a variable experience with the method, but it was generally agreed that a successful result was very uncertain and depended on factors little understood. I had few successful results in

following Romanowsky's method and just as many failures with Ziemann's procedures.

One turns with relief, therefore, to find that a modification recently suggested by Nocht furnishes a method which is invariably successful, without much dependence on the quality of dyes used, or the length of staining. Nocht's modification consists in the addition of a few drops of neutralized Unna's polychrome methylene blue (Grübler) to the 1 per cent solution of ordinary methylene blue. The usual specimen of polychrome methylene blue is distinctly alkaline and to be rendered effectual for the present purpose Nocht found that it required neutralization, preferably by acetic acid. This may be done by adding drop by drop of dilute 2 to 3 per cent acetic acid till the commercial fluid polychrome blue no longer turns red litmus-paper blue above the zone coming into immediate contact with the dye. I have never failed to secure a good result by the following procedure:

(1) To 1 oz. of polychrome methylene blue (Grübler) add 5 drops of 3 per cent solution of acetic acid (U. S. P., 33 per cent).

(2) Make a saturated (1 per cent) watery solution of methylene blue, preferably Ehrlich's rect. (Grübler), or Koch's, dissolving the dye by gentle heat. This solution improves with age, and should be at least one week old.

(3) Make a 1 per cent solution in water of Grübler's aqueous eosin.

The mixture is prepared as follows:

To 10 cc. of water add 4 drops of the eosin solution, 6 drops of neutralized polychrome blue, and 2 drops of 1 per cent methylene blue, mixing well. The specimens, fixed in alcohol or by heat, are immersed for two hours, specimen side down, and will not overstain in 24 hours. The density of the blue stain may be varied to suit individual preferences. The above proportions need not be rigidly followed, but the polychrome solution should be accurately neutralized.

Nocht later reports that the two solutions of methylene blue may be replaced by a 1 per cent solution of Ehrlich's rect. methylene blue, alkalinized by a few drops of ½ per cent of sodium hydrate, and left a few days in a thermostat at 50° C. This is the ordinary laboratory method of improvising polychrome methylene blue. With this preparation the procedure is as follows: To 2 cc. of water add 2 to 3 drops of 1 per cent watery eosin, and drop by drop of the alkalinized methylene blue till the original red color of the eosin has almost disappeared. In this fluid specimens stain in 5 to 10 minutes. I have had little success with this method.

The rationale of the Nocht-Romanowsky method is not yet fully un-

derstood, but it appears most probable that a staining agent which unites selectively with chromatin exists ready-formed in polychrome methylene blue, and may be developed in specimens of methylene blue in various ways, among which is slow digestion with an alkali and heat. Nocht refers to this agent as "red from methylene blue." It is not the commercial methylene red, but may be extracted from polychrome methylene blue, etc., by chloroform (Nocht), and it is a reasonable expectation that it can be put on the market in pure form.

Nocht's method furnishes so much information regarding the minute structure of the parasite and renders the identification of the parasite so complete and positive that it must be recommended above all others. Moreover, it has a large field of application in the study of nuclear structures in various other microorganisms.

Goldhorn (N. Y. Path. Soc., Feb. 13, 1901) has recently succeeded in digesting polychrome methylene blue with lithium carbonate, neutralizing with acetic acid, so as to develop in it a large proportion of the red-staining principle. His method is as follows: Fix the preparation, which must be fresh, by immersion in pure methyl alcohol for 15 seconds. Wash in water and stain 7 to 30 seconds in 0.1 per cent aqueous solution of eosin. Wash and stain in digested polychrome blue 30 to 60 seconds.

Besides rapidly staining the chromatin of the parasite, Goldhorn's fluid demonstrates most exquisitely the early appearance of extreme granular degeneration of the infected red cell, in which respect it is a distinct improvement over any stain yet devised. The fluid can be obtained from various dealers in New York City.

II. GENERAL MORPHOLOGY OF PARASITES.

The Tertian Parasite.

The *youngest form* of the tertian parasite seen in the red cell is identical in appearance with the spore of the parent rosette. It is a compact spheroidal, or slightly oval, or irregular body, about 2 μ in diameter. It shows an outer rim of basiphilic protoplasm enclosing a single large nuclear body, which is achromatic to methylene blue but stains readily in hæmatoxylin or by Nocht's method, and which is usually enclosed or accompanied by a clear achromatic spot, termed by Gautier "the milky zone" (Plate XXIX, Fig. 1). In the fresh condition these bodies are noticeably refractive, especially the nucleus,

change their position but rarely their shape, and are never pigmented. From the earliest period of infection the red corpuscle is often swollen, and exhibits advanced granular degeneration.

Within a few hours after the chill the parasite is usually found to have assumed a somewhat characteristic ring shape, which it commonly maintains in some definite form up to the presegmenting stage (Plate XXIX, Figs. 2-11). These ring-shaped bodies measure from 3 to 4μ in diameter, and the regular ring form is retained, without marked increase in bulk at any point, for 6 to 8 hours. Sometimes the ring is elongated, one arm reaching across the cell, while a thin bow persists. Occasionally the ring appears to unfold, and the parasite stretches clear across the swollen cell, with the nucleus at one end. The tertian ring is rarely as geometrical or delicate as is the æstivo-autumnal signet ring. The development of pigment was inconstant in the present cases, some large rings failing to show pigment, but usually one or more fine grains were to be seen in the medium-sized and smaller forms. The ring always encloses a considerable mass of hæmoglobin. The nuclear body of the tertian ring is its most characteristic feature, appearing as a rather large, achromatic, highly refractive body, after methylene blue, but staining intensely with hæmatoxylin and by Nocht's method.

Significance of the Ring Form.—In regard to the formation and significance of the ring opinions are at variance. Most of the Italian writers hold that the ring form is not really a ring, it being bridged across by a transparent and vesicular nucleus. There are many considerations favoring this view, especially the usual appearance of the parasite in the fresh condition, and the fact that the chromatin usually lies within the ring, eventually filling it. On the other hand, Mannaberg and Ziemann claim that this body is a true ring, formed by the thinning of the central portion of the body, and state that they have seen the ring develop in this manner in fresh blood. From the examination of the rings themselves I have been unable to convince myself as to which view is correct, but there are some early forms of the parasite which strongly indicate that the ring does not represent a vesicular nucleus. In one such form the ring is unfolded

and the nucleus lies naked in the hæmoglobin. Elongated forms of the young parasite are often seen in which the ring is absent and the nuclear body lies bare at one end. These forms vividly recall the appearance of *Amœba coli*, in which the nucleus remains at the hinder end during active movements of progression. Further, it is difficult to associate the relatively huge size of the ring with any nuclear structure, as this would require the young malarial parasite to have a nucleus which is larger than that of the adult *Amœba coli*. Again, secondary rings sometimes form from the union of pseudopodia, and these are identical in appearance with the primary ring but lack the chromatin granules (Plate XXIX, Figs. 6 and 8).

In specimens stained by Nocht's method the chromatin is usually found within the ring, sometimes lying in an isolated position in the centre, but very often the chromatin is found *outside* of the ring, connected by a very fine thread of protoplasm. If the ring represents a vesicular nucleus, we have here the anomaly of a complete separation of chromatin from the vesicular portion of the nucleus, which is opposed to some rigid histological principles. Even more frequently the chromatin is found to be enclosed by bluish staining protoplasm which shuts it off entirely from the ring (Plate XXIX, Figs. 7 and 8).

From various biological studies it appears that the nuclei of the protozoa are usually widely different from those of the metazoa; that many protozoa do not have a vesicular nucleus, with cell membrane, linin, nucleolus, etc., but possess the so-called "distributed nucleus" composed of a number of granules lying free in the body of the parasite. The study of the malarial parasite by Nocht's method indicates that the nucleus of this protozoon is of the *distributed type*, which does not exhibit a vesicular structure nor possess a nuclear membrane.[2] On these grounds I am inclined to agree with Mannaberg and others who hold that the form in question is a true ring—a form usually, but not necessarily, assumed by the parasite—and does not represent a vesicular nucleus.

Comparison of the Tertian and Æstivo-autumnal Rings.—From the study of the ring-shaped tertian parasite and the æstivo-autumnal

[2] See Calkins on "Protozoan Nuclei" in Annals N. Y. Acad. Sciences, 1898, xi, Part iii.

signet-ring forms, in typical cases of these infections, it appears to me that these parasites, with very rare exceptions, can be fully distinguished from each other, even in this early stage, by the following peculiarities:

(1) The nuclear body and chromatin mass of the young tertian parasite is achromatic to methylene blue, which densely stains the nucleus of the æstivo-autumnal organism. I have been unable to find in the literature any specific reference to this peculiarity as a diagnostic point, but it may be readily verified by comparing specimens of the two parasites stained by methylene blue and by Nocht's method. The dense staining of a nuclear body in the young æstivo-autumnal parasite has often been noted, and in 1894 Okintschitz mentioned the fact that the nucleus of the young tertian parasite fails to stain by methylene blue.

(2) The tertian ring is usually coarse and irregular, but the æstivo-autumnal ring is geometrically circular, more delicate, with an extremely fine bow, and usually with a typical signet-like swelling.

(3) One or two grains of pigment are almost invariably found in the early tertian ring, but are, with nearly equal constancy, absent from the æstivo-autumnal signet-ring.

(4) My specimens confirm the statement of Gautier that the tertian ring is usually pigmented before the chromatin becomes subdivided, while the chromatin of the æstivo-autumnal ring is always subdivided before the appearance of pigment. In some cases, however, the chromatin of the tertian ring divides before the appearance of pigment.

(5) The infected cell is usually swollen from the moment of infection by the tertian spore, and commonly shrunken when harboring the æstivo-autumnal ring.

All of these characters are usually apparent in ordinary specimens, but naturally are most distinct in flatly spread and rapidly dried corpuscles. I have encountered no exception to these rules in cases of infection by the tertian parasite in New York, and cases of æstivo-autumnal infection from Cuba. In many of the irregular relapses in cases showing tertian organisms encountered among volunteer

soldiers during the winter of 1898-99, single ring-shaped parasites not admitting of positive identification have frequently been seen. The significance of this observation will be considered later (see section on Plurality of Species of Malarial Parasites, p. 490).

After a period of 6 or 8 hours the tertian ring is usually found to have developed an outgrowth which is actively amœboid in the fresh condition and appears in stained specimens as a tongue-like protrusion or turban-shaped mass attached to one segment of the ring (Plate XXIX, Figs. 4 and 5). The nuclear body meanwhile increases slightly in size, projecting into the ring, and the chromatin divides into several large granules.

At this period occurs the greatest amœboid activity of the parasite, and in some severe tertian infections the parasite may be found fixed in the height of its excursions, when it presents in stained specimens the peculiar appearance depicted in Plate XXIX, Figs. 9 and 10. Here the ring is unfolded and the body of the parasite is strung out into a number of slender threads with nodal thickenings. At times the number and delicacy of the threads greatly exceed those seen in the sketch, and in some red cells the parasite may be found distributed in a series of fine granules between which the connecting threads are with difficulty distinguished. These latter are probably to be classed with "quinine forms."

A close inspection of corpuscles harboring such parasites often discloses the presence in one corpuscle of two distinct nuclear bodies, indicating the co-existence of two parasites. Frequently in one corpuscle are found twin parasites, entirely separate one from the other, each of which shows a tendency to develop the long threads (Plate XXIX, Fig. 9). When, however, the threads are numerous and very thin it is usually impossible to find any break in their continuity, while in many instances the two parasites are distinctly united. The significance of these two forms will be considered later (p. 475 et seq.).

During the second quarter of the cycle the body and the nucleus of the parasite develop rapidly in size, amœboid motion and amœboid figures gradually diminish, and pigment is abundantly deposited in the form of fine dark brown or yellowish grains showing in the fresh

state active vibratory motion. The infected red cell continues to increase slightly in size, its hæmoglobin is progressively diminished, and granular degeneration is extreme. Depending upon the character of amœboid activity the variety of figures seen during this period is very great. Eventually, toward the end of 24 hours or possibly somewhat later, the parasite occupies three-quarters of the swollen cell, in the form of a spheroidal or elliptical, homogeneous body, the outer portion of which contains most of the pigment and is rather more deeply stainable than the zone immediately surrounding the nucleus (Plate XXIX, Figs. 12 and 13). The nucleus gradually increases in size, growing into the ring. It no longer has the appearance of a small highly refractive achromatic spot (after methylene blue), but takes a light bluish tinge with 1 per cent methylene blue, and stains less deeply than before with hæmatoxylin. At the end of this period the nucleus completely fills the ring, stains rather distinctly with methylene blue and sometimes exhibits a delicate bluish network.

By Nocht's method the changes in the nucleus are found to consist in the gradual subdivision of the chromatin granules, which finally become rather numerous, of minute size, and more difficult to stain. Usually these chromatin granules lie on the inner circumference of the bow of the ring, projecting within the ring, and partly surrounded by a "milky" unstained zone. This milky zone is often absent in young parasites in cells thinly spread and rapidly dried, but in older parasites it is always present.

Various other positions may be assumed by the chromatin mass, as follows: a subdivision of the granules into two distinct groups, separated by a strand of bluish stained protoplasm; an eccentric position entirely apart from the ring; a position midway between two rings formed in the same parasite; a position in the centre of the ring entirely apart from any bluish protoplasm; a circular arrangement about the periphery of the ring. Sometimes the smaller granules are grouped about a central larger granule, as has been noted in other protozoa whose nuclei are of the "intermediate type" (*Microglena*, *Euglena*).

The third quarter of the cycle is occupied by the continued growth of the parasite in the form of a large homogeneous richly pigmented body, which finally occupies at least four-fifths of the swollen red corpuscle, and by certain nuclear changes which it is difficult to follow in specimens stained by methylene blue or hæmatoxylin, but which are fully demonstrated by Nocht's method.

The exact limits within which the parasite may be termed "full grown" can be sharply fixed only with great difficulty, but there appears to be a period of at least 12 hours, during which there is little change in the structure of the organism and during which the body stains homogeneously and the nucleus occupies the entire ring. This period may be placed between the 24th and 40th hour of the cycle. A portion of it is occupied by nuclear changes belonging to the reproductive phase of the parasite's development.

After the appearance of a faint intranuclear network most authorities agree that the nucleus largely disappears, so far as can be determined in specimens stained by methylene blue or hæmatoxylin, and that it is next seen in the form of highly refractive achromatic spots in the meshes of the reticulated presegmenting body (methylene blue), and these again stain deeply with hæmatoxylin.

Nocht's method, however, fully demonstrates the nuclear changes which occur in the full-grown parasite. A considerable area, usually the entire original ring, is now occupied by a "milky" or slightly bluish staining substance in which lie a considerable number of very fine chromatin granules. These granules are usually difficult to stain, and being of very minute size they are difficult to see. This fact has led Ziemann and Gautier to admit the possibility that the chromatin may actually disappear at one stage of the development, especially since they have found some large parasites in which no chromatin was demonstrable. In my specimens there were a very few large tertian parasites in which no chromatin granules appeared, but these were not more numerous than younger forms which were also devoid of chromatin and must therefore be regarded as sterile. I therefore interpret the larger forms devoid of chromatin as sterile forms, and cannot accept the view that the chromatin entirely dis-

appears at any stage of the fertile parasite, a view which is at variance with biological principles.

After the subdivision of the chromatin has reached a limit the next change, observed in a considerable number of parasites, appears to consist in the extension of a portion of the milky substance and its chromatin granules into the body of the parasite (Plate XXIX, Fig. 13.) At the same time the granules of chromatin increase in number. Other forms may be seen in which the "milky substance" and chromatin granules occupy an elongated space within the body of the parasite, and in such cases the beginning concentration of pigment and deeper stain of the parasite indicate the presence of the presegmenting stage (Plate XXIX, Fig. 14).

Presegmenting Bodies.—In specimens stained by methylene blue the first demonstrable indications of the division of the parasite are seen in *deeper staining capacity* and *tendency toward reticulation* which appear throughout the whole or in a part of the body of the parasite. Occasionally these changes may be noted in one-half the parasite while the other half retains the homogeneous appearance of the "full-grown" organism. Usually the process is found to have affected the entire organism, giving the very characteristic forms sketched in Plate XXIX, Figs. 15 and 16. In the presegmenting bodies the pigment is gathered in a reduced number of coarse grains, which lie in the body of the parasite in a position determined by that of the new multiple nuclei.

These bodies were first described by Golgi in fresh blood and properly interpreted as belonging to the process of segmentation. Later they were described by Marchiafava and Celli[3] as vacuolated parasites, the highly refractive nuclear bodies appearing in the fresh condition very much like vacuoles. Still later, Celli and Guarnieri sketched them from specimens stained in the fresh condition, regarding some as showing partial segmentation, others as vacuolated parasites, although they accurately described the appearance of the nuclear particles invariably found within these "vacuoles," while still others they supposed to be groups of confluent parasites, *i. e.* true plasmodia. Mannaberg's descriptions (1899), referring only to fresh blood, do not include these bodies, nor

[3] *Atti de R. Accad. med. di Roma*, 1887, 2. S., iii, p. 277.

have they found a distinct place in his plates, although some of the figures in his Plate IV indicate that they have not escaped his observation.

Thayer and Hewetson,[4] in their careful study of the parasite in fresh blood, designate as presegmenting bodies the parasites with collected masses of pigment (*corpi con blocchetto* of the Italian writers).

Laveran [5] refers to the similarity in appearance between a nuclear body and a vacuole, but he neither describes nor depicts the presegmenting reticulated parasite.

Ziemann [6] describes the presegmenting bodies as they appear after his or Romanowsky's staining methods, but the plates would not enable one unfamiliar with the subject to identify these forms in specimens stained by eosin and methylene blue.

The reticulated presegmenting tertian parasites were seen in every case of the present series examined within the 6 or 8 hours preceding the chill, and often in belated parasites shortly after the chill. Many transitional stages between the homogeneous adult parasite and the perfect rosette may be seen in rich infections. They are well demonstrated by eosin and methylene blue, especially as regards the increasing density of stain and the reticulation. After hæmatoxylin the multiple nuclei stain deeply by methylene blue.

By Nocht's method a series of interesting nuclear changes may be followed in the presegmenting forms. After the mass of enlarging chromatin granules and milky substance has flowed out into the elongated form described above, the chromatin granules leave the central clear space and make their way in groups out into the body of the parasite. Various stages of this process may be followed in specimens taken at suitable periods, and some observed phases are seen in Plate XXIX, Figs. 13-16. Considerable difference in the numbers of such groups may be noted in different cases. Usually a large number of ill-defined groups are seen, before the central mass of granules is exhausted (Plate XXX, Fig. 14). In one specimen the compact nuclei of the young spores appeared to form in one segment of the parasite before the main mass of granules had become exhausted.

[4] *The Johns Hopkins Hospital Reports*, 1895, v, p. 3.

[5] Traité du paludisme. Paris, 1898, p. 62.

[6] Ueber Malaria- und andere Blutparasiten. Jena, 1898.

Each of the groups appears always to be surrounded by a milky zone, and the mass of granules is often of a peculiar triangular form (Plate XXIX, Fig. 12). During these changes the pigment granules increase in size, diminish in number, and are distributed in the meshes of the now distinctly reticulated body of the parasite.

Tertian rosettes (Plate XXIX, Fig. 17) are usually seen in the circulation three or four hours before the chill, most abundantly just before the chill, and a few are often to be found for one hour or more after the chill. These limits may occasionally be much wider, as Marchiafava and Celli have seen rosettes 2 to 6 hours before the chill, and 6 to 7 hours thereafter; and indeed, when the different broods of parasites are not very distinct, there is no reason why occasional rosettes should not be found at any period of the main cycle.

Of the three types of sporulation described by Golgi, the second, according to which the entire parasite is divided into spores, leaving nothing but pigment, is undoubtedly the usual process. As regards Golgi's first type, in which only the peripheral portion of the parasite divides, leaving a distinct central globular pigmented body, most stained specimens fail to show convincing evidence that the physiological process of segmentation may be subject to such an important modification, nor does it appear in recent literature that the existence of this variety of segmentation has been fully verified. In a few specimens from patients taking quinine, I have seen rare segmenting bodies which resemble those described as above by Golgi, but never in fresh cases.

Golgi's third type of "partial segmentation," together with the "lateral circumscribed sporulation" of Celli and Guarnieri, may frequently be seen in rich tertian infections in fresh blood, but according to the evidence of stained specimens this must be classed with the presegmenting forms.

The tertian rosette is usually distinguished by its large size and considerable number of spores—fifteen to twenty. In the present cases it did not appear, however, that the identification of the tertian rosette could always be based upon the number of spores. Marchiafava and Bignami have described tertian rosettes with 40 to 50 spores.

With the smaller number of spores the rosette was usually much larger than either the quartan or the æstivo-autumnal body.

The nuclear changes demonstrated by Nocht's method in the tertian rosette consist principally in the gradual fusion of the new-formed groups of chromatin granules into single compact globules, which are partly surrounded by "milky zones." While the rosette is still compact the vesicular shape of the spore is distinct. The outer segment of the ring is usually thickened, the nucleus tends to lie near the inner pole, and between the nucleus and outer segment is a small "milky zone." The pigment is usually collected into a central block or mass of granules, but may be found variously scattered among the spores, or along the periphery of the rosette.

The Quartan Parasite.

The earliest form of the quartan parasite (Plate XXXI, Fig. 1), as seen in the stained red cell, is practically indistinguishable from that of the tertian organism, but its true character may usually be suspected from the slightly shrunken appearance of the infected cell. In fresh specimens the higher refractive quality of this parasite is often, however, sufficiently characteristic for its identification. After a very slight increase in size the quartan parasite becomes rather easy to distinguish in both fresh and stained specimens, for it usually remains smaller, more compact, and is more richly and coarsely pigmented than the tertian organism. As with the latter, the nuclear body is found projecting into the ring (Plate XXXI, Figs. 1-4). In fresh specimens at this period, the higher refractive qualities and slower amœboid motion are additional diagnostic characters.

The growth of the quartan ring is very similar in all important respects to that of the tertian, while its distinguishing features, especially the abundance of coarse pigment grains, are uniformly retained (Plate XXXI, Figs. 5-8).

During the presegmenting stage the characters of the quartan parasite are markedly different from those of the tertian. On account of the slower progress of sporulation, and from the greater tendency of the quartan parasite to complete its cycle in the general circulation, quartan presegmenting bodies are relatively much more numerous

in the stained specimens than are the similar forms of the tertian organism. In some specimens taken several hours before the chill the majority of organisms found may present the markedly reticulated structure indicative of approaching division. The multiple nuclei are less numerous, and the pigment is more abundant, and is often found in irregular, partly radiating rows. The coarsely reticulated, relatively small, and richly pigmented bodies lying in markedly shrunken cells are very characteristic and not readily confused with any other form of malarial parasite commonly found in the peripheral blood (Plate XXXI, Figs. 10-12). In some severe æstivo-autumnal infections, showing many parasites of all stages in the peripheral blood, somewhat similar spheroidal or presegmenting forms of the same general appearance may be found in considerable numbers, but as will be seen by reference to Plate XXX, the character of the pigment in the æstivo-autumnal parasite is very different. Such cases are very rare, and readily recognized on clinical grounds, being almost invariably of the pernicious type.

The quartan segmenting bodies are relatively more abundant in the peripheral circulation than are rosettes of any other variety of malarial parasite, and are easily identified by the small number (6 to 12) and comparatively large size and geometrical arrangement of the spores (Plate XXXI, Fig. 14).

The Æstivo-Autumnal Parasite.

The following description applies to a group of organisms which, according to the Italian school, comprises two or three varieties of malarial parasites. Waiving for the present the question of plurality of species, the entire group will be described as one, and the grounds alleged for their separation will be considered later. The present description rests upon the examination of some 260 cases of æstivo-autumnal infection occurring in U. S. soldiers who had shortly before arrived at Montauk Point from Cuba, and on a smaller number of cases seen in New York during the past few years. The conclusions drawn from the original examinations have been verified or modified by more careful study of these specimens during the past winter and also by the microscopical study of the tissues of a number of cases coming to autopsy.

The *earliest form* of the æstivo-autumnal parasite seen in the red cells, in the present cases, was very similar to that of the tertian and quartan parasites, but was slightly smaller than either, and was often distinguishable from the tertian by the shrinkage of the cell, and from the quartan by its distinctly smaller dimensions (Plate XXX, Figs. 1 and 2). In fresh specimens the young amœboid body usually showed a low refractive index as compared with the tertian and quartan parasites. It was never pigmented. Associated with the intracellular spores there were frequently seen in the plasma small spheroidal bodies, exhibiting an active rolling motion and occasional blunt projecting points (pseudopodia?), which, on becoming arrested by contact with red cells, were found to be indistinguishable from the intracellular bodies. Although seldom identified in stained specimens, it appears probable that these bodies were young extracellular forms of the parasite. The positive identification of these extra-cellular bodies, however, appears to be a very hazardous undertaking (cf. Ziemann, p. 49). In dried specimens stained by Nocht's method, however, the young extra-cellular parasite may be positively identified from the presence of a characteristic mass of chromatin. In my specimens such extra-cellular bodies were rarely encountered.

At a very early period of its development the æstivo-autumnal parasite in the present cases assumed a very characteristic *ring shape.* Many of these rings early developed a thickening of one segment, and to these bodies of various sizes the term " signet-ring " very aptly applies (Plate XXX, Figs. 5-7). It was noted that in some cases the rings failed to exhibit this thickening, but remained of a *uniform but very fine caliber throughout* (Plate XXX, Fig. 4). The period during which the rings retained this uniform caliber was not determined, but bodies of this type were seen measuring at least 3 μ in diameter. They nearly always presented two nuclear bodies, lying at opposite poles or close together. Occasionally such rings were found to have unfolded and to be stretched like a thread across the cell, the nuclei appearing at inconstant intervals. In other cases no rings of this type were seen, all showing the thickening of the signet and a single nuclear body. In the majority of cases rings of both types were associated in variable numbers.

No connection was demonstrated between the clinical features of these cases and the occurrence of these two types of rings, as both were found in cases showing intermittent tertian paroxysms, quotidian paroxysms, remittent fever of seven days' duration, and irregular fever for longer periods.

Multiple infection with the young rings was very common in the red cells of most cases of the present series and, as a rule, its occurrence was proportionate to the severity of the disease. In the peripheral blood three parasites were often found in the same red cell, occasionally as many as four; while in smears of the marrow of a fatal case infection of one red cell with four rings was common, five parasites were occasionally seen in the same cell, and one slightly swollen red cell was encountered containing seven well-formed rings (Plate XXX, Fig. 2). These observations accord with those of Ziemann (op. cit., p. 49), who found often three and four parasites, and once as many as five, in one red cell.

It appears in the descriptions of *Hæmamœba immaculata*, which is said to sporulate without producing pigment, that most of the rosettes contain comparatively few spores, averaging from 6 to 10 (Marchiafava, Bignami, Ziemann, Marchoux, Grassi and Feletti). The close resemblance to a non-pigmented rosette presented by some of these red cells harboring 5 or 6 young parasites was very striking. In my specimens (Plate XXX, Fig. 2) there could be no doubt as to the proper interpretation of the appearances.

Multiple infection of the red cell appears in rather rare instances to lead to the development of a peculiar form of the young æstivo-autumnal parasite on which Mannaberg bases his unique theory of the development of crescents. This body consists in the apparent union of two rings by a fusion of their nuclear bodies (Plate XXX, Fig. 3). Mannaberg depicts all transitional forms between these bodies and the fully developed crescent. Many examples of these double rings were encountered, but the various transitional forms from young double rings to crescents, shown in Mannaberg's plates, were not seen in the present cases.

The signet-ring forms frequently reached a diameter of 4 μ, while

still retaining the peculiar thickening of one segment and a very distinct nuclear body staining with methylene blue and surrounded by a narrow achromatic zone (Plate XXX, Figs. 6 and 7). Beyond this size, when persisting in the peripheral blood, the growth of the parasite produced an irregular body in which the outline of the ring became more or less obscure. The exact periods required in the development of these rings were not definitely determined, but in six cases taking quinine typical signet-ring forms were seen in the peripheral blood 60 to 72 hours after the beginning of the paroxysm. It seems probable, however, that these were belated individuals belonging to a somewhat scattered brood of which the majority either had retired to internal capillaries or had been destroyed by quinine. Yet in some cases in which the blood changes were followed at intervals of 6 to 12 hours, the increase in the size of the rings proved to be surprisingly slow, and the impression was obtained from these cases that the full development of the signet ring usually requires at least 24 hours and sometimes longer.

In the majority of cases the ring forms seen in the peripheral blood failed to show any trace of pigment, especially in patients showing distinctly intermittent quotidian or tertian paroxysms. In a considerable number of instances, however, especially in very severe and fatal infections, the largest rings exhibited a few very minute pigment grains, and were then usually associated with older pigmented forms.

The *later forms* of the æstivo-autumnal parasite are rather rarely seen in the peripheral circulation. Most of the Italian writers speak of their occurrence in the blood of the finger as being very unusual but not unknown. Sacharoff, in two cases of æstivo-autumnal infection, saw many rosettes in the peripheral blood. Ziemann reports that in malignant tertian cases occurring in Italy, he could follow, in the blood, the complete cycle, but that in cases occurring in Kamerun the later forms were not found in the finger blood. Plehn describes a variety of parasite which he believes is peculiar to hæmoglobinuric fever, and of which the later forms are of very small size but abundantly represented in the peripheral blood.

In five cases of the present series the entire developmental cycle of the æstivo-autumnal parasite could be followed in the peripheral

blood, and on the forms observed in these cases is based the present description of the later phases of this parasite.

After the ring has reached its full size (4 μ, 24 hrs. +), the swollen segment begins to increase in bulk and to involve a larger portion of the circumference, yielding forms seen in Plate XXX, Figs. 8-10. Some of these forms closely resembled the turban-shaped rings of the tertian parasite (Plate XXIX, Figs. 4 and 5), but were much smaller. A few fine pigment grains were usually found scattered along the periphery of the growing segment. Forms corresponding to the full-grown tertian parasite with homogeneous body were rarely seen in the peripheral blood of these five cases, but occasionally some were encountered (Plate XXX, Figs. 11 and 12) occupying three-fourths of the shrunken cell, staining homogeneously with methylene blue, and failing to exhibit a distinct nuclear body after methylene blue or hæmatoxylin. In sections of tissues from fatal cases these bodies appeared to be rather more numerous, but it was very difficult to distinguish in sections such homogeneous bodies from the slightly reticulated bodies representing the next stage of development. The fact that all other stages of the parasite were abundantly represented in the blood of these cases, while the homogeneous forms were very few, indicates that this period of development passes rapidly with the æstivo-autumnal organism.

Most of the larger forms of the parasite seen in the blood-smears of these five cases gave evidence of approaching segmentation, exhibiting a distinctly reticular structure and a condensation of pigment into one or two clumps (Plate XXX, Figs. 13 and 14). In many of these bodies the original ring persisted at one segment of the parasite, but appeared to be of reduced size and was sometimes subdivided by strands of protoplasm. The nuclear body at this period failed entirely to stain with methylene blue and was indistinct after hæmatoxylin, resembling in this respect the full-grown homogeneous tertian organism. The presence of a distinct achromatic spot adjoining the clump of pigment was very frequent in these forms and this spot was found by Nocht's stain to be composed of chromatin granules. The reticular structure of these bodies was usually distinct and the meshes were coarse.

The further development of the presegmenting forms is represented in Figs. 14 and 15, Plate XXX. In them the reticular structure becomes more distinct, the pigment is still further concentrated, and the subdivided nuclear bodies appear as small achromatic spots in the meshes of the reticulum and again stain distinctly with hæmatoxylin.

Rosettes (Plate XXX, Fig. 16) appeared in the peripheral blood of the five cases in moderate numbers and exhibited, in all, a very uniform structure. The pigment was grouped in a central granular clump, or, rarely, was somewhat scattered. The spores seemed to be arranged in two rows, but this appearance was probably an optical effect produced by the flattening of the more or less spheroidal body of the rosette, the spores originally lying in the central axis of the parasite falling, in the hardening process, within those lying in the periphery. When admitting of accurate enumeration their numbers were found to vary between 18 and 21. The same number of spores was repeatedly counted, in favorable specimens, smeared from the marrow of fatal cases. In sections of the tissue of fatal cases, however, the number of spores appeared to vary between wider limits, *i. e.*, 8 to 20, but as the entire rosette need not always be included in the section, the observations made in smears are the more reliable. A rim of hæmoglobin invariably surrounded the rosette and strands of hæmoglobin were frequently found running between the spores for a variable distance, sometimes within the outer row. These rosettes differed from tertian segmenting forms in the smaller size of the body and shrunken appearance of the cells, and in the small size, *but not in the number*, of the spores.

In none of the blood-smears nor in sections of tissues of the fatal cases were any rosettes seen without pigment. Although the arrangement of the spores and pigment often varied, there were no indications of the subdivision of the process of segmentation into the three types described by Golgi, nor were any forms seen which resembled the bodies described by Celli and Guarnieri,[1] and referred by them, with some uncertainty, to irregular sporulation.

The changes in the chromatin of the æstivo-autumnal parasite can be

[1] *Fortschritte d. Medicin*, 1889, vii, p. 528.

followed in specimens stained by Nocht's method (Plate XXX, Figs. 6-12), but on account of the smaller size of the parasite and the scarcity of older forms in the blood, it is difficult to trace the early phases of segmentation. In the young ring forms the early subdivision of the chromatin has been noted by Gautier, and in my specimens was a prominent differential character from the tertian rings. A great variety of appearances was produced by the irregular subdivision and distribution of the chromatin in the young æstivo-autumnal parasite, many of which have been sketched or described for the tertian rings. The grains were usually quite small and were sometimes apparently fused into a spindle-shaped mass, lying within the ring. Other peculiarities noted were: a markedly unequal size of the grains, a widely separate position, a frequent concentration in the centre of the ring, and, very rarely, a complete absence of chromatin.

After 24 hours' growth, the chromatin granules became more numerous and extremely minute, and were enclosed in a trace of the "milky substance," as in the tertian parasite.

When any considerable quantity of pigment gathers in the æstivo-autumnal parasite it is usually found in one or two groups, but rarely is diffuse. When the parasite has reached the full-grown homogeneous stage the pigment is commonly found concentrated in a single compact mass. This early concentration of pigment is one of the chief features which distinguish the æstivo-autumnal from the tertian parasite in the presegmenting stage. This fact has been fully emphasized by Gautier and was very uniformly illustrated in my cases. The changes in the chromatin in the presegmenting æstivo-autumnal body (Plate XXX, Figs. 13-15) are similar to those of the tertian parasite. In some of the specimens the chromatin granules were found in radiating lines stretching from the parent mass to the new peripheral groups (Plate XXX, Fig. 13). In many specimens the peripheral group of granules was well formed while the central portions of the body contained many diffuse granules (Plate XXX, Fig. 14). The relative quantity of chromatin in some of these bodies appeared surprisingly large. The spores in the mature rosettes usually contained single compact grains of chromatin (Plate XXX, Fig. 16), which stain readily by methylene blue, but in some rosettes

two large granules of chromatin were seen in a few spores, although the rosette seemed ready to burst. The double nuclei seen in many young æstivo-autumnal rings may perhaps be referred to the incomplete fusion of the chromatin in the rosette.

III. ON THE PLURALITY OF SPECIES IN THE ÆSTIVO-AUTUMNAL GROUP OF PARASITES.

The probability that several species of parasites are concerned in the severe types of malarial fever prevailing in warm climates, especially during the summer and autumn, has been maintained chiefly by Marchiafava, Celli, Bignami, and Grassi. From their studies of this group of fevers they divide the æstivo-autumnal group of parasites into two species: (1) The quotidian, and (2) the malignant tertian.

1. *The quotidian parasite.*—The typical fever-curve of this variety the authors found rather rarely, more frequently in relapses than in initial seizures, while a postponement of paroxysms was usually observed, and a continuous fever was very common. The typical attack is short, the fever lasting 6 to 8, rarely 12, hours, the temperature then falling to 37° C. or lower. The descriptions of the morphology of this parasite unfortunately refer only to the appearances in fresh blood.

During the rise of the temperature, the sweating stage, and the first hours of apyrexia, the blood was found to contain a variable number of red corpuscles infected with one or more very actively motile, or non-motile, parasites of discoidal or ring shape. During the afrebrile period the parasite increased in size, the amœboid motion diminished or ceased, and fine pigment grains were deposited along the periphery of the organism. Later, in the larger forms, the pigment gathered in a single clump or heap of grains. During the entire development the infected red cell diminished in size and presented a "brassy" color as a result of "acute necrosis" induced by the parasite. Rosettes were seldom encountered in the blood of the finger, segmentation occurring principally in the internal organs, as seen in the aspirated splenic blood. Rarely segmentation occurred before pigmentation, but usually the numerous round or oval spores were found grouped about a central pigment mass, the rosettes being much smaller than those of the quartan or common tertian parasite. Contrary to the rule in malignant tertian infections, the young parasites were found in the blood from the beginning of the paroxysm, and, except in very mild cases, there was no

period in the cycle when the parasites were absent from the blood of the finger.

2. *The malignant tertian parasite* is distinguished by the authors on both clinical and morphological grounds. Clinically the typical paroxysm begins with a sharp elevation to about 40° C., the febrile period lasts 24 or 36 to 40 hours, is marked by a pseudo-crisis and pre-critical elevation, the fever describing, in the three-hourly chart, a characteristic course which differs from that of the mild or common tertian paroxysm. A tendency towards various irregularities is common.

In the blood the parasites may be scarce or even entirely absent at the beginning of the paroxysm. At the height of the fever the red cells contain certain small, non-motile, annular or disk-shaped bodies, or irregular amœboid bodies, which begin to show pigmentation towards the approach of the afebrile period. Most of the parasites then disappear from the peripheral blood, and rosettes are rarely seen except in some very rich infections. The presegmenting forms are round or ovoid, are one-fourth to one-half the size of the red cell, and the pigment is gathered in a single clump or in a mass of vibrating granules. The rosettes occupy about two-thirds of the red cell and exhibit two rows of spores which usually number 10 to 12, rarely 15 to 16. The infected cells are markedly shrunken and present a "brassy" or "golden" appearance.

The authors distinguish the malignant tertian parasite from the common or mild variety on the following features:

(1) The malignant tertian parasite is smaller in all stages. (2) It assumes the ring shape, which, in the benign tertian parasite, is never seen. (This statement has been shown by many writers to be erroneous. Most of the young, mild tertian parasites are ring-shaped.) (3) Its pigment is less abundant and often non-motile, while in the other the pigment is very abundant and always in vibratory motion. (4) The rosettes are smaller, contain only 10 to 12 (rarely 16) spores (?), and are rarely seen in the finger blood. (5) The infected cell is shrunken instead of being swollen, as with the mild tertian infection.

From the quotidian parasite the malignant tertian is distinguished on the following grounds:

(1) The malignant tertian amœba is, in corresponding stages, larger and less transparent than the quotidian. (2) In the tertian parasite the amœboid movement is livelier, so that the resting discoidal forms are less frequent than with the quotidian parasite. The larger pigmented tertian forms also are often amœboid, this property persisting for 24 hours or longer. (3) The pigment of the tertian parasite is often

vibratory, but never in the quotidian. (4) In the quotidian rosettes pigment is sometimes wanting. (5) The appearance in the finger blood of a new generation of tertian parasites is seen some hours after the beginning of the paroxysm, therefore much later than with the quotidian infection.

Marchiafava and Bignami admit that the similarity between the malignant tertian and the quotidian parasites is very great, and that the differential diagnosis is very difficult, and possible only from the full-grown forms seen just before the paroxysm. They apparently do not feel quite certain that the quotidian and malignant tertian parasites are separate species, as is indicated by the following extract from their discussion on this point:[8]

"The remarkable points of resemblance between the quotidian and malignant tertian parasites make it very difficult to solve the question whether we have to do with different sorts of parasites in the strict sense, or with one and the same parasite which varies greatly in the time of its development—24 to 48 hours—and there are all intermediate degrees. On this latter theory it becomes easy to ascribe the morphological differences to the varying length of the cycle. But various facts oppose this hypothesis. First, the clinical types of the quotidian and tertian are clearly distinct from each other, and have a certain stability which is maintained in relapses and recurrences. Second, we have never met with intermediate forms or transitional cases, although it is very difficult to interpret the irregular fever. Granting that the question cannot at present be solved definitely, . . . we are inclined to adopt the view that the amœba of the quotidian and the amœba of the summer tertian are closely related varieties of one and the same parasite."

Mannaberg[9] accepts the views of Marchiafava, Celli, and Bignami, in respect to the separate nature of the malignant tertian and a quotidian group of parasites, and his description of the morphology of the parasites does not differ from that of the preceding authors whom he largely quotes. From his description of the single case of quotidian fever it is impossible to determine how many groups of parasites were present in the blood.

Grassi and Feletti[10] include all tertian parasites in one class (*Hæmamœba vivax*) and state that an easily recognized species of malignant quotidian parasite (*H. præcox*) is found in Catania during the sum-

[8] Translation, *The New Sydenham Society*, London, 1894, vol. cl.

[9] Die Malariakrankheiten. Wien, 1899.

[10] *Centralbl. f. Bakter.*, 1891, x, pp. 449, 481 and 517.

mer and autumn. Their opinion appears to be based largely on an acceptance of the views of Marchiafava, Celli, and Bignami, as they offer no detailed description of these parasites nor of the cases in which they have found the latter variety.

Of the cases reported by Thayer and Hewetson, in 114 young æstivo-autumnal parasites were seen in the blood, and of these cases 73 exhibited quotidian fever. Although the examination of these cases was made almost wholly with fresh blood, it is significant that they found no evidence on which to subdivide the group of æstivo-autumnal parasites.

Ziemann, from the study of 210 cases of æstivo-autumnal infection, was unable to find sufficient grounds for the subdivision of this group. He, however, mentions the fact that the small parasite observed in cases occurring in Crema and Grosseto showed slightly less amœboid motion than that found in cases in Kamerun, and admits the possibility that quotidian æstivo-autumnal fever may be referable to a parasite which completes its development in 24 hours. He regards it as equally probable, on the other hand, that the quotidian fever is caused by the growth of two generations of small tertian parasites, but in his entire series he was unable, on account of the disappearance of the parasites from the peripheral blood, to determine accurately the length of the cycle. In his cases of malignant tertian fever, the prolongation of the febrile paroxysm, described by Marchiafava and Bignami as a characteristic feature of the malignant tertian infection, was not always to be seen. On these various grounds he concludes that all forms of the æstivo-autumnal parasite belong to one group of which the cycle varies in length from 24 to 48, or possibly 72 hours.

Gautier,[11] who has very carefully studied the malarial parasites of the Caucasus in specimens stained by Romanowsky's method, has failed to find any which he could regard as completing their cycle in 24 hours. Gautier's charts illustrating the forms of the parasite prevailing in the blood at various periods of the cycle very graphically illustrate the difficulty of following the development of the æstivo-autumnal parasite in the peripheral blood. The prolongation of the febrile paroxysm was sometimes present, sometimes wanting, and appeared to be referable to the maturation of sub-groups of parasites (see his Curve III).

In my cases there was a moderate number of pure tertian paroxysms caused by infection with a parasite morphologically identical

[11] Ueber den Parasit Laveran etc. (Russian). Moscow, 1896, and *Ztschr. f. Hyg.* 1898, xxviii, p. 439.

with the malignant tertian of Marchiafava and Bignami. The prolongation of the paroxysm and the pseudo-crisis were sometimes observed. I encountered the same difficulty experienced by Ziemann and Gautier in determining the length of the cycle from the parasites in the peripheral blood, and believe that it is seldom possible on this evidence alone to demonstrate a 48-hour cycle for the parasite. The infecting brood is seldom very compact, and in rich infections, in which alone presegmenting bodies and rosettes appear in the blood, the groups are nearly always multiple. In my preliminary report [12] on the Montauk cases this difficulty was noted.. Indeed, in 6 cases, it seemed impossible to find any marked change in the size of the parasites in the peripheral blood for three days after the chill, the ring forms persisting for that period and being constantly supplied by constantly maturing rosettes in the visceral capillaries (see also Gautier's tables). The frequency of cases showing the ring forms persisting, with slight changes, for two or three days, led to the belief that the cycle of development must sometimes extend over 48 hours. I was not then familiar with Golgi's conclusions, but since find that this observer, who is certainly qualified to know what evidence is needed to establish a 48-hour cycle, concluded that "the parasites in the blood of æstivo-autumnal cases are only an index of the infection, have little to do with the real pathogenesis of the fever, and that they represent early phases of a cycle which is much longer than has been believed."

In one of my cases, however, in which the examinations of the blood were supplemented by microscopical examination of the viscera, a 48-hour cycle appeared to be demonstrated. For the present purpose, therefore, the temperature chart furnishes by far the most convincing evidence.

The majority of my cases, however, showed quotidian excursions, and the temperature chart was of little value in determining the length of the cycle. In these the parasites in the blood, and occasionally in the viscera, were submitted to microscopic examination in the fresh condition, and in specimens stained by eosin and methylene blue, and by Nocht's method. From this study, only one feature was noted

[12] *New York Medical Journal*, 1899, lxix, pp. 114 and 149.

which could possibly serve to separate the parasites into two groups, and this related to the form of the young rings. The majority of the young æstivo-autumnal rings exhibit a single chromatin granule and a distinct thickening of one segment of the ring, and this latter character is maintained from the very smallest form up to bodies at least 4 μ in diameter. In addition to these signet-ring forms, other rings were seen which lacked the signet, were provided with two chromatin granules usually located at opposite points in the circumference of the ring, and which retained this appearance up to a considerable size (3 μ, rarely 4 μ). Such rings have been repeatedly observed before and accurately sketched (Gautier, Ziemann, Marchoux). See Plate XXX, Fig. 4.

In some severe cases these rings, lacking the signet, constituted the majority of all those seen in the blood; usually both forms were abundantly present, and in some distinctly tertian cases, while the signet-rings were more numerous, the other type was also represented in small numbers. I am, therefore, unable to conclude that these peculiar rings belong to a separate species of parasite with a short cycle of development.

No other morphological differences were noted in the young rings of these cases. It is possible, but hardly probable, that the quotidian parasite, if it exists, was not represented among the cases seen at Montauk.

Between parasites of larger size, as seen in the blood and in smears of the viscera of fatal cases, considerable difference in size was noted, but the smallest presegmenting bodies and rosettes encountered were found associated only with the usual form of signet rings. My observations on the parasites in the fresh condition failed to show any uniform difference in the refractive quality or amœboid activity, but under the circumstances they could not be pursued so extensively as was desired.

The evidence secured failed therefore to establish any clinical or morphological grounds on which to separate the parasites of pernicious malarial fever into two or more groups.

It does not seem likely that such a division can be successfully maintained except on morphological grounds. The difficulty in fol-

lowing the development in the blood of even a 48-hour parasite is well illustrated by Gautier's tables, and must be considerably increased when dealing with a more rapidly maturing form. The tendency of the æstivo-autumnal parasite to be held in the viscera during its later phases very greatly complicates the undertaking. In the cases of quotidian infection reported in detail by Marchiafava, Bignami, and Guarnieri, one fails to find, in the results of the blood examination, convincing evidence of the existence of one group of parasites with a 24-hour cycle. The clinical peculiarities observed in these cases are not without significance, but are of themselves entirely inconclusive and still require confirmation. The evidence on which the quartan, tertian, and æstivo-autumnal parasites are separated is of entirely different value from that on which it is proposed to divide the æstivo-autumnal group. Distinct morphological characters have not been clearly established, peculiar clinical features have not been shown to characterize any considerable group of cases, the developmental phases of a 24-hour cycle have not been demonstrated in the blood or viscera, and it would seem that further observations are required before the existence of a quotidian parasite can be accepted even as a working hypothesis.

The later position of Marchiafava and Bignami, admitting that the existence of two species in the æstivo-autumnal group is not proven, would therefore seem to be justified.

That 72 hours may occasionally be required for the cycle of the æstivo-autumnal parasite is indicated by the observation of a few cases of quartan fever with this infection. Such cases have been reported by Gautier and Ziemann. The paroxysms, in Ziemann's case, were twice repeated, with intervals of two days; the fever almost completely subsided in the interim; and there seems to be no reason to suppose that the irregular maturation of tertian broods could possibly have produced the paroxysms.[13]

[13] R. Koch, whose report on malaria in tropical countries has appeared since the completion of this article, has also reached the conclusion that there is but one species of the æstivo-autumnal parasite, and that in fresh cases the fever is uniformly of the tertian type, but later tends to become more and more irregular. He considers "tropical" a more appropriate epithet than "æstivo-autumnal" to designate this parasite and the fever caused by it (*Deutsche med. Wochenschr.*, 1900, p. 781).

Hæmamœba immaculata.

Grassi and Feletti, Marchiafava and Celli, Bignami, Guarnieri, Sacharoff, Marchoux, and Ziemann, report cases in which rosettes free from pigment were found in the blood or viscera. Most of these authors, while admitting that the parasite may occasionally sporulate without producing pigment, are not inclined to regard *Hæmamœba immaculata* (Grassi) as a separate species.

Grassi and Feletti, however, claim to have observed in a bird pure infection with a variety of parasite which failed to produce pigment, and regard the appearance in the human subject of rosettes without pigment as evidence of infection by a distinct species of parasite. Mannaberg also accepts this view.

In the report of the examination of the viscera of this bird by Grassi and Feletti no mention is made of the presence or absence of pigment, and it is impossible to determine whether or not the infection had failed to produce pigment in the viscera as well as in the peripheral blood. In all cases in which pigment-free rosettes have been found in the blood of human subjects there have been found the usual pigment deposits and pigmented rosettes in the viscera. That there is considerable variation in the quantity of pigment produced by the parasite in fatal cases is shown by the reports by Marchiafava and Bignami of fatal cases in which a microscopic examination was required to show the presence of very scanty deposits in the viscera. Ziemann mentions in this connection that he has seen a presegmenting body of the benign tertian type which was entirely free from pigment.

I have already mentioned (p. 447) the marked resemblance which red cells, harboring five or six parasites, may bear to pigment-free rosettes. Most of these rosettes, as described, contained a small number of spores (6 to 10). In the sketches of Marchiafava and Celli, as noted by the authors, the spores of the pigment-free rosettes are of unusually large size. Their appearance is almost identical with the cell harboring seven young parasites sketched in Plate XXX, Fig. 2, from the marrow smears of a fatal case. If the latter cell had been found in a section of tissue it would have been scarcely possible to distinguish it from a rosette without pigment.

In a drawing accompanying the article of Bastianelli and Bignami,[14] is a nearly normal red cell apparently infected with six young parasites, in explanation of which the authors suggest an irregular form of segmentation. If the drawing is accurate, it appears to me that the cell

[14] *Bull. d. r. Accad. med. di Roma*, 1894, xx, p. 151, plate i, fig. 26.

is too little altered to have long harbored a growing parasite, and that the young parasites are too large for spores. The drawings of the cerebral capillaries, filled with rosettes without pigment, hardly admit, however, of this interpretation. Nevertheless, a failure of the human malarial parasite to produce pigment is such a violent departure from its ordinary physiology that the fact should rest only on the most absolute proof, and it may not be amiss to have suggested a possible source of error in this field.

In any case the grounds are insufficient to warrant the classification of *Hæmamœba immaculata* as a separate species of parasite, and seem at best merely to justify the opinion of other observers that pigment-free rosettes, as seen in the human subject, are an occasional form of the æstivo-autumnal parasite. This opinion is well set forth in the words of Marchiafava and Bignami:[15] "We cannot allow that a distinction should be drawn between the *Hæmamœba præcox* (tertian parasite of Grassi) and the *Hæmamœba immaculata*, as two separate species. Segmentation with no pigmentation has been observed by Marchiafava and Celli, but only in very rare cases, and always together with pigmented rosettes. So that in these cases it would be necessary to suppose a double infection, an hypothesis that is devoid of all foundation. We shall feel unable to change our opinion until we meet with cases which show no trace of melanæmia and which, therefore, mean a pure infection with the *Hæmamœba immaculata*."

IV. THE NUCLEAR BODY OF THE MALARIAL PARASITE.

From the earliest period of the minute study of the malarial parasite certain structures in its body have been recognized as probable nuclear elements, but the exact significance of these structures and their relation to the definite nuclear elements of metazoan cells have never been fully determined.

In 1889, Celli and Guarnieri,[16] from the examination of fresh malarial blood stained by methylene blue in ascitic fluid, described in the larger parasites, an outer deeply staining ectoplasm and an inner nearly achromatic endoplasm. In the lightly colored endoplasm, surrounded by a narrow, perfectly achromatic zone, was a sharply marked body of variable structure, sometimes compact, sometimes reticulated, but evi-

[15] New Sydenham Society's Translation, op. cit.

[16] References to the authors here cited will be found in the monographs of Thayer and Hewetson, of Ziemann, and of Mannaberg, already quoted.

dently representing the nucleus of the parasite. On the inner border of the ectoplasm of younger parasites they found a deeply staining body which they regarded as the early form of the nucleus. Their plates accurately depict the growth of the "endoplasm and nucleus" up to the presegmenting stage, into which they were unable to follow it. This demonstration of the nuclear body was, in the minds of competent observers, the beginning of the end in the controversy regarding the truly parasitic nature of the malarial organism.

Using the same technical methods, Grassi and Feletti, in 1890, described in the larger parasites "a large vesicular nucleus such as is seen in many rhizopods." This nucleus was usually eccentric, possessed a very thin, often indistinct membrane, and an intranuclear network filled with a semi-fluid substance. The intranuclear network exhibited a nodal thickening resembling a nucleolus, which was sometimes round or often showed several radiating filaments stretching toward the nuclear membrane. This nucleus was not found in young parasites. The authors believed they could discover evidences of direct division of the nucleus, beginning 12 to 16 hours before segmentation of the body. The nucleus of Grassi and Feletti undoubtedly corresponds to the endoplasm of Celli and Guarnieri.

In 1891 Romanowsky published his observations on the structure of the tertian parasite as demonstrated by his special staining method. He described the nucleus as a colorless central area in the parasite, in which appeared a smaller body staining of a carmine violet color, the "nucleolus" or chromatin of the nucleus. In the larger parasites he described the development of fibrillar chromatin bodies in the nucleus, indicating a process of indirect division. These filaments were indistinct but gave the mass of chromatin a less compact appearance. The diaster stage is roughly indicated in one of the sketches. Romanowsky described, also, "quinine forms" in which the clear zone of the nucleus was wanting, this structure fading insensibly into the body of the parasite, while the chromatin was subdivided into many fine granules. To judge from the drawings, these "quinine forms" appear to be identical with Gautier's presegmenting forms (see p. 440). Romanowsky mentions no stage of the parasite which failed to show a nuclear body stainable by his method.

Sacharoff, in 1891, observed the "nucleolus" lying in a clear nucleus in specimens of æstivo-autumnal parasites stained by gentian violet, and noted the disappearance of the nucleus just before segmentation. In 1893 he applied Romanowsky's method to the minute study of the nucleus and described a fibrillar structure which occasionally showed karyo-

kinetic figures. He found, further, that the flagella of the parasite stain like the chromatin of the nucleus, and concluded that the flagella are separate chromosomes of the karyokinetic nucleus, extruded from the parasite under the influence of cold. In 1895, he reported a further study of the nucleus, and described the "extrusion of chromosomes" (exflagellation) from the malarial parasites of young crows. The parasites of these animals were found specially adapted to the purpose, as their nuclei are large, chromatin filaments are distinct, and flagellate bodies are found in the blood immediately after shedding. In these parasites he depicts intracellular formation of flagella and the extrusion of all the chromatin from the body of the parasite in the form of flagella. The author refers (1895) to the studies of Sala on the eggs of *Ascaris megalocephala*, in which indications were found of an active movement on the part of the chromosomes, and to the conclusion of Strasburger that the changing position of chromosomes in some vegetable cells results from an active movement on the part of these structures. It may be added that in recent years an extrusion of all chromatin in the form of flagella has been observed in various forms of coccidia by several investigators.

Bastianelli and Bignami described the minute structure of the æstivo-autumnal parasite in specimens stained by hæmatoxylin. In the young parasite they describe as "endoplasm" the large central achromatic area through which shines the hæmoglobin of the infected cell, while the deeply staining peripheral granule was said to consist of chromatin. This nuclear body, or endoplasm, possesses no membrane and exhibits no special structure. The chromatic granule increases in size as the parasite develops and the clear endoplasm acquires a light bluish tinge, partly obscuring the hæmoglobin. Later, when the pigment has gathered in a single mass, the body of the parasite becomes homogeneous and the chromatic granule disappears. These changes mark the beginning of the reproductive phase, and may be followed very shortly by segmentation. During segmentation the chromatin reappears scattered through the body in fine particles, about each of which a ring of chromatophilic substance gathers. A small remnant of the endoplasm is left unutilized in the segmenting process. The spores at first contain no endoplasm, which appears only in the young parasite. The authors do not find, either in their own preparations or in the drawings of others, any definite structures recalling a true nucleus. The disappearance of the chromatin before sporulation they find to be analogous to a similar phenomenon in the Gregarinidæ and Coccidia, while in the Oscillariæ one or more disseminated granules of chromatin represent

nuclear bodies similar to those seen in the malarial parasite. The nucleus of the malarial amœba, they believe, never assumes the vesicular or resting stage on account of the rapid succession of generations. In the crescents, with rare exceptions, they found no chromatin, and therefore regarded these bodies as sterile.

Mannaberg (1893, 1899) followed the development of the nucleus, as described by Celli and Guarnieri, in specimens stained by hæmatoxylin, and by a special procedure of his own. He was unable to find evidence of a karyokinetic division of the nucleus.

Ziemann studied the structure of the tertian, quartan, and æstivo-autumnal parasites by means of his modification of Romanowsky's stain. He followed minutely the changes in the nucleus in each variety, and described many minor variations which may be of value in differential diagnosis. At a rather early period of the cycle the solid chromatin granule was usually found to become less compact and was sometimes divided into two or three portions. With the disappearance of amœboid motion in the parasite the chromatin is usually divided into many fine filaments or spindles from which are derived, during the full-grown and presegmenting stages, an increasing number of secondary chromatin bodies of variable position and contour, but eventually forming the nuclei of the young spores. He found the eccentric position of the nucleus in the young tertian and its central position in the quartan parasite to be very constant differential characters in these organisms. In the "full-grown" stage of the parasite he found the chromatin more difficult to stain. The parasites which failed to exhibit a mass of chromatin he regarded as sterile. He first described appearances which recalled the chromatin filaments and mitotic figures of Romanowsky, but later concluded that no distinct traces of a true karyokinetic process could be demonstrated in these parasites. After a growth of 16 to 24 hours, the chromatin mass was found to break up into a number of spindle-shaped granules, which showed a very inconstant arrangement. Meantime the limits of the nucleus became very indistinct. In many parasites the nucleus and chromatin disappeared, the parasite increased markedly in size and presented a rich deposit of pigment grains in active vibratory motion. These forms he regarded as sterile, and included among them the elliptical and large oval bodies, in most of which he was unable to demonstrate any traces of chromatin. In a few crescents obtained from the bone-marrow, 11 hours after death, he was able to demonstrate a more or less compact mass of chromatin, but always of reduced bulk.

Okintschitz has described the nucleus of the young forms and the fine

structure of the malarial parasite in specimens stained by eosin, methylene blue, and safranine. In the young parasite the nucleus was found to be a compact mass which, in the tertian parasite, failed to stain with methylene blue, but in the æstivo-autumnal variety stained densely with this dye. The further changes in the nucleus were not fully traced.

Marchoux describes the nucleus of the æstivo-autumnal parasite of Senegal in specimens stained by eosin and methylene blue and by a mixture of thionin and carbolic acid (see p. 431). In the early ring-shaped organism the enclosed substance was regarded by the author as the nucleus, the deeply staining eccentric body as the nucleolus. Sometimes two nucleoli were found at opposite poles of the parasite, an appearance which he was inclined to refer to a process of conjugation in view of the fact that later phases exhibited a single nucleolus. In the full-grown stage the nucleolus assumed a position in the centre of the nucleus, gradually dividing into a number of smaller bodies arranged in the form of a wreath. The later changes were not followed, as the parasites disappeared from the peripheral blood.

Gautier, in 1895, reported a study of the malarial parasite of the Caucasus in specimens stained by Romanowsky's method. He finds that the nucleus consists of a vesicular portion and a violet-staining mass of chromatin. The chromatin body is usually surrounded by a narrow, "milky zone," which is sometimes continued about the entire vesicular nucleus. In the ring stage the hæmoglobin shines through the vesicular portion of the nucleus. In many parasites at various stages the "milky zone" is invisible. With the beginning enlargement of the body of the parasite the chromatin changes from a small compact body to a less compact oval mass of granules. It sometimes early breaks up into two or three portions, or it may consist of a single mass of small granules. In some of Gautier's drawings these granules are placed in the centre of the hæmoglobin mass which he regards as shining through the vesicular nucleus. In bodies probably representing the early presegmenting stage of the parasite he describes the development of a reticular structure of the parasite and the total disappearance of chromatin particles. Later the chromatin grains reappear in the meshes of the reticulum. In crescents and ovoids he found numerous small chromatin granules. In the crescents these granules appeared to be much more minute than in the ovoids. Many large parasites without chromatin he regarded as dead.

The nuclear changes which I have observed in specimens stained by Nocht's method have been detailed under the descriptions of

species. They were largely in accord with the observations of Gautier and of Ziemann.

I find that the nucleus of the parasite belongs to the "distributed type" of protozoan nuclei (p. 436), consisting of granules of chromatin and, certainly in the older and possibly in all stages, of an achromatic substance in which the granules are embedded. While the claim of Bastianelli and Bignami must be admitted, that the parasite possesses "no true nucleus," in the metazoan sense, it exhibits nevertheless all the nuclear structures required in some protozoa.

Neither the nucleus nor the achromatic substance appears to be necessarily connected with the interior of the ring, which is the form assumed by the young and vegetative parasite. It seems most probable that this form represents a true ring, or if not, the ring is bridged by a substance which has no essential nuclear relations.

I find no forms in the fertile cycle of the parasite in which chromatin cannot be demonstrated. Various intra-cellular and extracellular forms devoid of chromatin are for that reason necessarily regarded as sterile.

Although there appears to be abundant analogy in the nuclear changes in the parasites of birds and in some closely related coccidia, to indicate that the human parasite may divide by a modified form of karyokinesis, I could find no sufficient ground for applying this term to the series of nuclear changes observed in the presegmenting parasites in man.

Labbé, Danilewsky, Sacharoff and others, in the blood parasites of animals, and Simond and Siedlecki, in various coccidia, find that the chromatin regularly appears at some stages in the form of fibrils, and that these may describe figures rather closely resembling the mitoses of metazoan cells. Romanowsky claimed to have seen distinct chromatin filaments, and sketches imperfect mitotic figures in the tertian parasite. Ziemann relinquishes a similar claim in his second article, admitting that no distinct mitotic figures are to be demonstrated in the human parasite. Gautier's sketches show nothing of these filaments. In my specimens from fresh malarial blood chromatin was never seen in the form of a filament, all elongated masses being invariably of granular structure. On the other hand, when exflagel-

lation occurs with the human parasite, the chromatin becomes filamentous, figures resembling monasters are produced, and the chromosomes are extruded as active flagella. This process is entirely in accord with the changes depicted by Sacharoff in the parasites of birds.

It appears, therefore, that in the fertile cycle of the malarial parasite division occurs by a very simple process which may be likened to amitosis, the only visible changes in the chromatin being subdivision and fusion. In another cycle of development, adapted for the extracorporeal growth of the parasite, division occurs by a modified form of karyokinesis, the chromosomes leaving the parent cell to fertilize other individuals. Some of the structures seen in coccidia by Simond and Siedlecki have been interpreted as showing the fertilization of one parasite by the flagellum of another. MacCallum,[17] working with the blood of infected crows, repeatedly saw free flagella enter other parasites in which they became lost, and he was able to repeat this observation in a case of human æstivo-autumnal infection. Evidence is therefore gradually being gathered to determine the true significance of flagellation and to locate in the proper place the function of karyokinesis in the malarial parasite.

V. THE CRESCENTIC BODIES.

While the results of recent studies of the coccidia (Simond, Siedlecki, Schaudinn) bear on some obscure points in the biology of the malarial parasite, the full significance of the crescentic bodies, even in the coccidia, has not yet been demonstrated, although the position of these bodies in the developmental cycle has been determined. In various coccidia it has been shown that there are two cycles of development, one, the sporulating cycle, leading to the development of encysted bodies, the other, asporulate and parthenogenetic, leading to the development of crescentic and flagellate bodies. The individuals of the sporulating series are capable of reproduction in the host, but in the asporulate series, the crescents and flagellate forms are very fragile, disappearing rapidly when exposed to unfavorable conditions, and alone are incapable of self-perpetuation. There is evidence in the coccidia that some of the crescentic bodies represent the female element and require fecundation by the flagellum or male element, in order to become fertile.

[17] Journal of Experimental Medicine, 1898, iii, p. 117.

MacCallum's observations on Halteridium, a crescentic parasite of birds, indicate that these crescentic bodies are of two varieties, one, the male, producing flagella, the other, the female, uniting with a free flagellum and developing into a motile form called the "vermiculus."

Further evidence on this point has been furnished by Ross, who found that when the blood of birds infected with Proteosoma, a species of parasite closely resembling the malarial amœba, reaches the stomach of the mosquito, many of the organisms become flagellated. A few days later he finds in the stomach-wall of the mosquito certain large encysted pigmented bodies containing many rod-like structures and some "black spores," which on the rupture of the cyst gain the general circulation. In the salivary glands of the insect these "germinal rods" may be found in large numbers. Ross was able to infect young birds by subjecting them to the bites of mosquitoes fed on blood containing Proteosoma, but was unsuccessful with Halteridium.

Grassi, Bignami, and Bastianelli, have confirmed and extended Ross's observations. These investigators succeeded in conveying the æstivo-autumnal infection from one human being to another by means of a particular variety of mosquito, Anopheles claviger, the "dapple-winged" mosquito described by Ross. Moreover, they fed their mosquitoes on blood containing crescents, showing that these bodies are capable of further development in a new host. In the mosquito they observed, as Ross had done, exflagellation of the crescents, development of encysted bodies in the stomach-wall, discharge of "germinal rods," and their accumulation in the salivary glands of the infecting insect. Later they succeeded in transferring the tertian parasite in the same way, the large hyaline forms furnishing the flagella in the mosquito's stomach. They found no bodies resembling the vermiculus of birds, and it has not been shown how the parasite pierces the wall of the stomach. It is thus clear that the crescentic body is a form of the parasite adapted to further development in a new host.

Of the mode of origin of the crescents in man there is still nothing definitely known. In support of Mannaberg's theory that they are conjugation forms resulting from the union of two ring forms, no new facts have been observed. Grassi and Feletti, who, in 1891, claimed that in birds a certain number of crescents were undoubtedly produced by conjugation of younger forms, have apparently not insisted upon the correctness of this view. On the contrary, the studies of the development of coccidia in the rabbit, salamander, cuttle-fish, and other animals, strongly oppose Mannaberg's theory, for in these organisms, which, according to Metchnikoff, are clearly related to the malarial parasite, the

crescentic form is produced in an entirely different manner, by the segmentation of a large spheroidal body into several small but fully formed crescentic bodies. No similar parent bodies have been described in the blood or tissues of the human subject.

Celli and Guarnieri, in 1889, and Canalis in 1890, depicted the young forms of the crescentic bodies as small, narrow crescents with considerable fine pigment, lying within slightly altered red cells, and traced the development through a gradual increase in size, with destruction of the hæmoglobin of the infected cell, up to the adult crescent. From the adult crescents, ovoid, elliptical, and spheroidal bodies may then form, and these frequently become flagellated. These phases of development have been very generally accepted, and are largely in accordance with analogous processes in various coccidia. There is, however, a lack of agreement regarding the relation of the ovoid and spheroidal bodies to the crescentic forms. When examined in the fresh condition crescents are frequently seen to assume the spheroidal form, and if a little moisture is added the spheres may extrude flagella. Occasionally, however, the spheres or ovoids may be seen to revert to the crescentic form, as described by Ziemann and others. Now, crescents of almost any size may be made to assume the spheroidal form, from which it appears that this body is not always to be included in the natural developmental series of the crescent. I have seen spheroidal bodies develop from crescents about which there was hardly a trace of hæmoglobin, while in other cases the spheroidal body did not occupy more than two-thirds of the red cell. The young crescents which appear in the blood on the fourth or fifth day of the paroxysm have, in my cases, been of small size, rather broad, and often no longer than the red cell. They are often distinctly oval or spheroidal in the stained specimen. During the fifth to the seventh days they gradually increase in size, with progressive destruction of hæmoglobin, and finally assume the elongated crescentic form, without hæmoglobin. My conclusion, therefore, is that the ovoid and spheroidal bodies seen in the ordinary stained specimen are usually younger forms than the elongated crescent, and that the spheroidal bodies which form in shed blood may be derived from crescents of almost any age. The quantity of hæmoglobin about the spheroidal

body would seem to be a reliable indication of the age and original form.

In 1889 Canalis described a form of *segmentation* in crescents. The segmenting bodies were elliptical in form and discharged eight or ten rather large spores. At the same time, Celli, and Marchiafava and Golgi, were inclined to believe that crescents might sporulate in the blood, but were not certain that they had ever seen such forms. Antolisei and Angelini, in 1890, confirmed Canalis's observations, stating, however, that the new spores possess a double contour. Grassi and Feletti, and Sacharoff, believing that the crescents represent a separate species of organism (*Laverania malariæ*), accept of necessity the hypothesis of their sporulation, but have not positively identified segmenting forms in the blood. Lewkowicz (189?) reports that he has seen two crescents in the act of sporulation in the blood, and in the splenic blood he describes some segmenting crescents containing as many as thirty spores. The transverse subdivision of crescents has been observed by Grassi and Feletti, Mannaberg, Ziemann, and others. Ziemann, however, regards this process as unquestionably not of a reproductive nature. The transverse segmentation of crescentic bodies has been clearly demonstrated in coccidia (Jackson Clarke), but the fate of the segments is not shown, and while there are indications that the crescentic bodies of coccidia may be multiplied to some extent in this way, the same evidence clearly shows that the process is exceptional.

The various phases of gemmation and budding described by many writers have never been strongly urged as a natural method of reproduction of crescents.

Accordingly, opposed to a moderate number of inconclusive and often uncertain observations favoring the segmentation of crescents, there are entirely negative results from the vast majority of observers.

Various studies of related protozoa indicate that crescentic bodies, after fertilization, regularly proceed to further development with encystment and the production of an entirely different form of the parasite, but sometimes leading to autoinfection of the same host. Although there is no satisfactory evidence that the malarial crescents can develop further in the human being, it is by no means certain that their formation and development are entirely innocuous to the patient.

Not a few observers have connected certain febrile paroxysms with the growth of crescents. Golgi in 1889 referred some forms of fever at long intervals to the development of new broods of crescents. Laveran still (1899) holds that crescents alone, without the presence of other forms, can be associated with a febrile paroxysm. Canalis, who claimed to have found sporulating crescents, connected the segmentation of these bodies with paroxysms recurring in three or four days. Celli and Sanfelice, and Grassi and Feletti not infrequently observed paroxysms in birds associated with the appearance of crescents only in the blood. Lewkowicz believes that the development of crescents may produce quotidian or tertian fever, or paroxysms at long intervals. He believes, also, that crescents are not so refractory to quinine as is generally supposed; that they disappear under quinine after a variable period, and that the long-persisting forms are new individuals reproduced in the viscera from day to day.

Most authorities, however, prefer to attribute the irregular paroxysms to the production of a limited number of ordinary amœboid parasites, which sometimes fail to reach the general circulation in demonstrable numbers.

The possibility that the development of crescents is associated with a febrile paroxysm is by no means disposed of by the proof that crescents do not sporulate. There is almost certainly a secondary cycle of development of the parasite leading to the formation of crescents, and this cycle may well be several times repeated, each time with fever. For each crescent destroys a red cell, and the crescents in the blood are sometimes as abundant as the brood of young amœbæ. Although most cases of fever at long intervals are probably simple relapses, it is impossible to deprecate wholly the tendency to regard some of these paroxysms as evidence of a second cycle of development in the parasite leading to the formation of crescents. In the Montauk series I was frequently surprised to find only young crescents in the blood associated with mild seizures at irregular intervals. It was especially noted in some cases in which crescents persisted in the blood after fever had subsided and while quinine was still being administered, that shortly after a mild chill numerous young crescents appeared in the blood.

Some *morphological features* of the crescentic bodies are still of

active interest. The early observers, who believed that crescents were an encysted form of the parasite, frequently depicted these bodies with a distinct double contour, representing a sharply defined membrane. Without entering into the details of opposing views, it may be said that this extreme claim of a distinct double membrane has been slowly abandoned, and the most that is maintained is the existence of a condensed outer border about the crescent. I find that this outer border may be colored red by strong staining with eosin. That the reddish border thus developed is not identical with the membrane of the red cell appears from the fact that it invests the concave side of the crescent where it may be widely separated from the projecting bow of the red cell. The remnant of the red cell which stretches like a bow across the concavity of the crescent, while usually single, appeared double in one of the specimens. The ends of the bows then overlapped, each enclosing a little more than one-half of the crescent (Plate XXX, Fig. 23).

The identity of the bow with the membrane of the red cell has been accepted without question, and its development in young specimens leaves little doubt of this origin, but why it should increase in dimensions with the growth of the crescent and why it is occasionally double are at present obscure questions.

The application of Nocht's method to the crescentic bodies furnished valuable additions to the knowledge of these forms. Ziemann found the vast majority of crescents to be entirely free from chromatin. Gautier, however, working with Romanowsky's procedure, was apparently the first to demonstrate the presence of chromatin in any large proportion of crescents in human blood. In the young ovoid or elliptical forms he found a well-marked group of rather large granules of chromatin. In the full-grown crescent a single group of very fine granules lying in the centre of the body and often partly obscured by the pigment mass, could, in the majority of specimens, be fully identified.

With Nocht's method I have been able to demonstrate chromatin granules in the vast majority of crescents in all stages (Plate XXX, Figs. 17-23). In the younger forms the granules were usually larger and more distinct than in the older forms. In the adult crescents the chromatin was usually found in a single rather compact mass of

minute granules, which was usually much obscured by overlying pigment. Occasionally the chromatin mass lay to one side of the wreath of pigment, in which case it was very easily identified. In a few specimens in which the pigment was diffusely scattered over the crescent, the chromatin was very clearly visible (Plate XXX, Fig. 22).

Two groups of chromatin granules were seen in a moderate number of crescents, in some of which the pigment was arranged in the form of the figure 8. In these specimens both groups of chromatin granules were inclosed in a single mass of clear achromatic substance (Plate XXX, Figs. 21 and 23).

In a few specimens it was impossible to detect any traces of chromatin, indicating that these particular forms were sterile. Such forms, however, were not more numerous than were chromatin-free parasites in ordinary cases of tertian or æstivo-autumnal infection, while they were difficult to find in specimens in which the staining had been especially successful.

Nocht's stain very clearly demonstrates an elliptical relatively achromatic area in the centre of the crescent in which the pigment and chromatin are usually included. The line of demarcation between the bluish staining poles and the achromatic area was often very sharp after the application of this method. Occasionally the achromatic area was found well out in one pole (Plate XXX, Fig. 20).

The application of Nocht's method to other forms of the parasite greatly increases the number of forms in which evidences of approaching segmentation may be found. In my specimens of crescents no variation in the chromatin granules or mass was detected pointing to a reproductive process. The older and larger the crescent the smaller and less distinct these granules became. In some spheroidal bodies of a fatal case, however, the body of the parasite was distinctly reticulated, although the chromatin grains remained in a single mass.

VI. EXTRA-CELLULAR PARASITES.

That the young parasite during its passage from the parent rosette to the new red cell is sometimes caught in the plasma in both fresh and dry specimens is evident from the reports of various observers.

The possibility of identifying such young free forms in the fresh

condition may, however, be doubted. Ziemann, commenting on this point, says that "only in the beginning of his studies of malaria did he venture to identify young amœboid organisms in the plasma of fresh blood." Celli and Guarnieri (1889) have sketched the appearance of young extra-cellular bodies, including forms of at least two species of parasites, in fresh blood stained by methylene blue after their special procedure, but many of these, especially the pigmented ones, were undoubtedly separated from the cell during the manipulation. In preparations of fresh blood, parasites so frequently pass from the cell into the plasma that it may be doubted if any accurate estimate of the number of extra-cellular forms in the circulating blood can be obtained by this method of examination. In some cases in which I have seen suspicious extra-cellular bodies in fresh specimens, Nocht's stain failed to show any extra-cellular parasites whatever. If reliance be placed upon dry specimens stained by this method, and the demonstration of a distinct nucleus be required, extra-cellular parasites must be looked upon as a comparative rarity, but may undoubtedly be seen in exceptional cases. Gautier and Ziemann depict such forms, while mentioning their rarity. Romanowsky could find young extra-cellular tertian parasites only in patients taking quinine. The same rule appears to hold with the later stages of the parasite, extra-cellular parasites being found with extreme rarity. In fresh specimens a considerable number of large forms appear to be extra-cellular, but these, in stained specimens, usually show some enclosing remnant of a red cell.

Various *sterile forms* of parasites described at length by Golgi, Ziemann, Bignami and Bastianelli, and others, while usually endoglobular, are sometimes seen in the plasma, and in dry specimens may be found to be distinctly extra-cellular. The characters of these sterile forms are, according to Ziemann: (1) increase in size, (2) loss of amœboid motion, (3) greater abundance of pigment and increased vibratory movement of pigment granules, (4) markedly hyaline appearance in stained specimens, (5) complete or nearly complete absence of chromatin. Ziemann, however, includes among the sterile forms crescents and spheres derived from crescents, which do not properly belong in this class, as they contain chromatin and under

suitable conditions are capable of further development. Other sterile forms described by Ziemann were found most abundantly in the splenic pulp after death. These were spheroidal bodies, of large size, hyaline aspect, vibratory pigment, and deficient supply of chromatin.

Similar large forms have often been described as derived from full-grown quartan and tertian parasites, and their extrusion from the red cell has been followed in specimens of fresh blood. The extra-cellular position of most of these forms appears therefore to be artificial. Working principally with dry specimens, I have always had great difficulty in finding any of these large extra-cellular forms, and believe that they are extremely rare in the circulating blood. In specimens of fresh blood, however, which have been allowed to stand for 1 to 24 hours such forms become rather numerous.

Vacuolation has also been frequently described in these large sterile parasites. In the earlier observations of parasites in the fresh condition the nuclei were sometimes mistaken for vacuoles, an error against which Golgi warns. In stained specimens I have very rarely been able to identify vacuolated parasites and believe that their identification in fresh blood is usually very hazardous.

The relation of the parasite to the red cell still remains a matter of dispute. Laveran holds that the majority of parasites are merely attached to the surface of the cell, though some are found within its substance. The crescentic bodies he regards as strictly intra-cellular.

The frequent appearance of the projection of the æstivo-autumnal ring beyond the circumference of the red cell has led many to believe that this parasite, at least in its early stages, is merely attached to the cell. Marchiafava and Bignami, however, point out that the æstivo-autumnal ring in the fresh condition never sends pseudopodia beyond the edge of the cell, and may be seen dipping down or swimming at different levels in the cell.

Gautier very accurately depicts the appearance of the ring projecting beyond the cell, and there seems to be no good reason to doubt that such parasites are merely attached to the cell. It by no means follows, however, that later stages of the æstivo-autumnal ring are not found within the cell, as described by Marchiafava and Bignami. It is generally accepted that the tertian parasite lies within the red cell,

yet in many tertian cases the body and especially the nucleus of the parasite appear to project beyond the border of the cell, even more distinctly than in the case of the æstivo-autumnal ring. Such attached forms seem to be more frequent in the actively amœboid stage, and in cases taking quinine. In fresh specimens which have been allowed to stand and are afterwards dried and stained, the parasite may be found in various stages of extrusion, and similar appearances of projection of body and especially of nucleus beyond the cell are numerous. Mannaberg probably correctly expresses the facts in this matter as follows: "The young parasites swim in the plasma for a very short time and soon become attached to red cells. They remain attached to the cell for a time but soon penetrate within, where their further development is completed."

It is probable that the æstivo-autumnal parasite remains attached to the cell longer than the tertian, possibly because it is less actively amœboid.

VII. ON A FORM OF CONJUGATION OF THE TERTIAN MALARIAL PARASITE.

In four cases of tertian infection I have encountered appearances in the blood which seem to admit of no other explanation than that of conjugation of malarial parasites. In a considerable number of other cases similar appearances were found, but much less frequently.

The blood in these cases showed a moderate number of young rings and a large number of half-grown and full-grown forms. A great many red cells showed double infection with young rings. In many instances these rings were entirely separate, each exhibiting a single large granule of chromatin. Many cells, however, contained two rings, which were clearly *fused together along one segment of the ring*, and two large chromatin granules were then invariably found at different points in the rings (Plate XXXII, Figs. 2-5). The fused parasites usually differed in appearance. One was a large delicate ring with a thin bow, and chromatin granule of moderate size, while the other was a coarser body with thickened bow, enclosing little or no hæmoglobin, and exhibiting a large chromatin granule (Plate XXXII, Figs. 3-5). These differences between the two conjugating parasites could not always be found. Among the single rings, the

two forms of young parasites were often distinguished, but no single rings could be found containing two *equally* large chromatin granules, while every red cell that exhibited two large and equal chromatin granules contained also two distinct rings. It appeared therefore that the bodies of many parasites had become fused together, while their nuclei remained separate. Occasionally the two chromatin granules were found close together, but no distinct signs of a fusion of chromatin were found at this stage.

On examining the parasites in later stages of development, most of them were found to have lost the ring form, and to have spread out into a large number of threads, with nodal thickenings, variously curled in the red cell. These threads evidently represented the pseudopodia of a very active amœboid stage. The chromatin masses were now subdivided into 10 to 12 granules, but in the majority of the cases these masses were far apart and showed no tendency to unite. In many cells, however, the amœboid figures were less marked, *and the masses of chromatin lay side by side united by a little achromatic substance.* Later some parasites were found in which the two groups of rather large chromatin granules lay in *immediate apposition, surrounded by achromatic substance.* This phase was marked by a distinct reduction in the length of amœboid figures (Plate XXXII, Figs. 6-11).

Many older, spheroidal, hyaline forms, belonging to this same brood, were found in these cases. All the older hyaline forms were single and exhibited a single large group of fine chromatin granules. Not one cell harboring two full-grown parasites could be found in prolonged and repeated search through several slides.

The question therefore arises, what became of the very large number of twin parasites seen in all the younger stages? In one of the Montauk cases the two broods were of different ages, one approaching segmentation *and all single*, the other less than half-grown and *almost invariably twinned.* Can twinning occur in part of a brood and not in its oldest members, or in one brood extensively and not at all in its predecessor? While such physiological variations are possible, they appear extremely improbable, and one is forced to the conclusion, merely from the absence of older twinned parasites, that conjugation

occurred. Whatever interpretation may be placed upon this peculiar absence of older twinned forms, the finding of all stages of union, first of the bodies, later of the nuclei, as illustrated in Plate XXXII, appears to admit of no other explanation than that of conjugation.

The further examination of these and other specimens developed some other peculiarities of interest. Single parasites of each of the above types could apparently be traced through later stages of development. The small, coarse, densely staining body remained comparatively compact throughout its development. It was rarely found in distinct ring form, enclosing hæmoglobin, but often exhibited coarse amœboid processes. It was usually of smaller size than the average tertian parasite, but the infected red cells were swollen and pale. In the full-grown stage this body was compact and densely staining, with rather distinct chromatin granules, but I could not trace it up to a sporulating body (Plate XXXII, Fig. 13). In many respects these large forms resemble the quartan parasite, but the infected red cell is swollen, the pigmentation is not marked and the majority of them, in younger stages, have been found to conjugate with the ordinary tertian rings.

The other type of parasite of the conjugating pair also frequently developed singly, but I am not certain that it reached sporulation. The young forms showed the delicate ring shape with thin bow (Plate XXXII, Figs. 3-5). The larger rings enclosed much hæmoglobin and often exhibited amœboid figures. The infected cells were distinctly swollen and pale. The full-grown form stained very slightly and appeared hyaline, while its chromatin was slight in quantity and minutely subdivided. No presegmenting bodies could be found in these cases which appeared to show the characters of this pale full-grown parasite. All presegmenting bodies and rosettes were either densely staining (before distinct reticulation), or of large size and with abundance of chromatin, the former developing from the compact forms described, the latter apparently from the conjugating parasites but possibly also from the single rings. Possibly the pale hyaline full-grown forms with finely subdivided chromatin were destined to become flagellate forms (compare MacCallum's two varieties

of crescents). Some of the above features are illustrated in the accompanying drawings (Plate XXXII).

Some considerations which do not favor the belief in a process of conjugation require mention:

1. The suggestion naturally arises that the presence of two masses of chromatin does not necessarily mean the presence of two parasites in one red cell.

From a long series of observations on the character of the chromatin in young tertian parasites I must admit that this objection is partly valid. The young tertian parasite, in some cases, may be found to contain two masses of chromatin. In the young compact body (mentioned above) (Plate XXXII, Figs. 1 and 3) these granules when present are large and of nearly equal size, but in the delicate tertian ring I have never seen two distinct and equal chromatin granules. In somewhat rare instances the ring shows an accessory granule of small size in the neighborhood of the main granule, but never, in my observation, have two large granules occurred in a single thin ring-shaped tertian parasite. The significance of these double granules is not clear. The appearance of two, very small, compact, spore-like bodies partly fused together, as may occasionally be seen, indicates that such forms may sometimes result from the early union of the bodies of two very young parasites. The accessory granules in thin ring forms have always appeared too small to have been derived in the same way. It seems probable that such accessory granules may result from the incomplete fusion of the original granules which go to form the chromatin of the spore, or, in other instances, from a precocious subdivision of chromatin in the young parasite, as suggested by Ziemann.

The presence of two nuclei in some very young compact parasites before they enter upon the process of conjugation with the large rings explains the occasional appearance of *three nuclei* about to unite, as seen in some conjugating forms toward the completion of the process (Plate XXXII, Figs. 9 and 10). In one case, the small single compact forms with two nuclei, and large conjugating forms with three nuclei were present in considerable and about equal numbers. That the presence of three, large, entirely separate, subdivided nuclei in

one conjugating form means the union of three original parasites, I do not believe; but the morphological appearances above described indicate that it invariably means the union of at least two parasites.

It appears, therefore, that the presence of two large and equal masses of chromatin in one infected cell indicates, with few exceptions, the presence of two parasites. Rarely three nuclei are seen in conjugating forms, two of which may be derived from two very young compact forms uniting very early, and the third from subsequent conjugation with a thin ring-shaped form.

The further development of young parasites with small accessory chromatin granules may be followed in rare instances. The accessory granule divides as does the main mass of chromatin and later unites with the other to form one clump of granules in the full-grown stage. Throughout these stages the total bulk of these two masses appears not to exceed the average for single parasites, whereas in the conjugating forms the excessive quantity of chromatin in all stages is a very striking feature. In all the examples of such single parasites that I have seen, the unequal size of the chromatin masses was distinct, and there were no appearances suggesting the presence of two parasites in the same cell. These forms, therefore, differ entirely from the conjugating forms above described. I have never seen more than two masses of subdivided granules in a single parasite, whereas three large and equal masses may be observed in conjugating parasites.

A third minute granule may rarely be seen, however, in young rings. Ziemann[18] describes the appearance of multiple chromatin masses in young tertian parasites. He was at first uncertain whether this appearance was referable to the presence of two fused parasites or to an early division of one nucleus, but finally accepted the latter explanation. He describes the separation of one, or rarely two, accessory granules from the original mass in cells infected by single parasites. Sometimes the accessory granule was much smaller than, sometimes nearly as large as, the main granule. All of these appearances I have seen in single parasites, less often in

[18] Ziemann, *Centralbl. f. Bakter.*, 1897, xxi, p. 643.

single members of conjugating pairs, and I agree with Ziemann as to their significance, but the conjugating forms above described are quite different, and do not appear in Ziemann's descriptions.

2. It may be objected, further, that it is impossible to determine when the bodies of two parasites are really united, as one may overlap the other and produce a false appearance of union.

This difficulty is undoubtedly present with some of the young forms, but with others the appearances of the parasites toward the completion of the process, when amœboid motion is subsiding, are, on the contrary, absolutely convincing that the bodies are actually fused. The significance of two large masses of chromatin surrounded by one achromatic zone is also unmistakable (Plate XXXII, Figs. 9 to 11).

3. Again, it may well be pointed out that examples of twin parasites of advanced development, presegmenting bodies and rosettes, are sometimes seen in severe tertian infections, furnishing examples of twinning when conjugation does not occur.

This fact is a matter of common observation, and in my series there are a few cases in which it was especially noted. In one red cell a typical rosette with many spores and a compressed hyaline body without apparent nucleus were observed. In another distended cell were seen one perfect rosette, one imperfect presegmenting body, and one compressed hyaline form.

It may be said of these twins, which proceed to segmentation without conjugating, that they are vastly less numerous than the conjugating forms or young twins seen in the same or other cases. I have, for instance, seen hundreds of conjugating forms within the past few months, but I remember only three or four twinned rosettes seen in as many years.

In the cases showing twinned adult parasites a few younger couples were seen, which showed no attempt to conjugate. The great majority of these young parasites and all the young twins in these cases were the typical tertian *ring-shaped* parasites, while the small compact forms were exceedingly hard to find. My observations on this latter point, however, are not so numerous as is desirable and are still in progress.

4. Finally, the comparative absence of older twinned parasites may be referred to the death and extrusion of one of the twins while the other proceeds to full development alone.

In some gregarines in which multiple infection of cells and conjugation of parasites is common (*Klossia*), one of the parasites often succeeds in dwarfing its companions and alone reaches full development. The dwarfed or dead parasites are then found in the cell alongside the growing form (Wolters, Clarke). In some instances of multiple infection by full-grown or segmenting malarial parasites, I have sometimes seen evidences of compression and death of the younger of two or three organisms. More often both parasites appeared to be equally favored. In any case, the remains of the dwarfed parasite ought frequently to be found if one member of the pair commonly inhibits the growth of the other. In the four cases referred to above, no traces of dwarfed parasites could be found, and while young twins were extremely numerous, all the older parasites were single. It therefore appears impossible to explain the entire absence of older twinned parasites, and especially of traces of any abortive individuals in these cases, on any other ground than that of conjugation.

I find, therefore, that the usual fate of twinning of tertian parasites is conjugation; that twins sometimes grow to maturity without conjugation, for reasons which are not clear, but apparently when both parasites show the usual ring form; that the union sometimes involves three parasites but probably always requires the presence of one or more compact densely staining forms, which do not commonly assume the ring shape, and of one of the typical tertian rings.

A further inquiry relates to the uniformity with which conjugation occurs, and its position as an essential or as an accidental phenomenon in the progress of malarial infection.

It would seem that a process so fundamental as the conjugation of individuals, if it occurs at all, ought to be an invariable feature of every active infection, but there is not sufficient evidence on which to base any such claim. The four cases referred to as furnishing numerous clear examples of conjugation were selected on account of the abundance of the conjugating forms, but in many other cases less

numerous though equally distinct examples were seen, indicating that the process is of very frequent occurrence. On the other hand it must be admitted that the majority of specimens from routine cases fail to show any distinct traces of the process; from which it may be concluded that conjugation is probably not an essential feature of the growth of the parasite.

In the four marked cases the infection was unusually rich, one of them showing more numerous parasites than I had ever seen before in benign tertian infections. One patient had just arrived at Montauk from Cuba, in September, two others were suffering from a first attack, in July, in New York City. In one case there was a prompt relapse a few days after quinine was omitted. It is possible that important clinical features may be found to be associated with the presence of conjugating forms, but the observations are too limited to furnish any conclusion on this point.

In æstivo-autumnal infections, in which twinning is very common, I have been unable to trace the parasites through the conjugating period on account of their disappearance from the peripheral blood. Of five cases showing rosettes and presegmenting bodies in the blood, in one were found several twins of these older forms in the same red cell, while in four others no twin parasites were found beyond the ring stage, in which evidences of conjugation, as described by Mannaberg, were occasionally seen. The presence of double nuclei in peculiar æstivo-autumnal rings has been noted. Marchoux suggests that such forms result from conjugation, an explanation which appears reasonable but which is at present without proof. Another more probable explanation has already been mentioned (p. 452).

I have been unable to secure any recent specimens of quartan parasites.

Double rings with fused nuclei are apparently a common form of the young parasite of Texas cattle fever (Theobald Smith).

VIII. ON THE PLURALITY OF SPECIES OF MALARIAL PARASITES.

The belief in a plurality of species of human malarial parasites has been accepted probably by a majority of clinical observers residing in temperate climates, but seems never to have gained uniform sup-

port from those who have studied largely in tropical climates, nor from comparative biologists.

The doctrine of plurality of species is maintained by Mannaberg, Koch, and the great majority of German writers, by Welch, Osler, Councilman, Thayer, Dock, and practically all American writers, and by Golgi, Grassi, Bastianelli, and Bignami, representing the Italian school. A middle ground is held by Kruse, Canalis, Marchiafava, Celli, and Sanfelice, Babes and Georghiu, Danilewsky, and Ziemann (in his early publications), who are inclined to accept the unicist theory, or claim at least that the facts do not warrant the belief in the existence of distinct species; while Laveran, Metchnikoff, Marchoux, Vincent, and some others actively uphold the existence of a single polymorphous species.

Among the pluralists no uniform basis of classification has been established. Those who rely strictly upon the morphology of the human malarial parasites rather uniformly agree upon the existence of three species—quartan, tertian, and æstivo-autumnal. Grassi and Feletti, influenced by the morphology of similar parasites in lower animals, add a fourth distinct species, *Laverania malariæ* (yielding the crescentic bodies), as well as *Hæmamœba immaculata*. Golgi regards the distribution of the parasite in the body of quite as much importance as a ground of classification as its morphology, and therefore makes two groups, one, including the quartan and tertian parasites, which are found principally in the peripheral blood, and a second, the æstivo-autumnal, which is found principally in the internal organs. Mannaberg regards the presence or absence of syzygia, *i. e.*, crescentic bodies, as the chief ground for the separation into species, and recognizes as species which do not produce crescents, (1) the tertian and (2) the quartan parasites, and as those which by conjugation form crescents, (1) the malignant tertian, (2) the pigmented quotidian, and (3) the unpigmented quotidian parasites.

Van der Scheer and Plehn, working extensively in India and Africa, find only two well-distinguished species, (1) the large and (2) the small forms. The former include the quartan and tertian parasites, the latter, the æstivo-autumnal or tropical group of other authors.

It is seen that while there are no distinctly contradictory views among the pluralists, there is an entire lack of agreement in regard to the grounds required for the separation of species. Since there is no room to doubt that a certain stability exists in the three generally recognized species of parasites, practically the question at issue is

whether these species are ever interchangeable, and, if so, to what extent and under what circumstances may one species pass into another. Mannaberg has fully presented the evidence in favor of a plurality of species, without, however, considering many opposing facts, and Laveran has ably supported his own belief in a single species, disregarding much contradictory morphological evidence. While it is unlikely that the question will be fully settled until the extracorporeal form of the parasite has been fully traced, there are some recent observations on the subject which may be profitably reviewed.

The strongest evidence in favor of a plurality of species is found in the results of experiments on the inoculation of malaria, which, when properly controlled, have invariably produced the type of organism found in the specimen of blood used in the inoculation. Mannaberg tabulates 33 experiments of this nature, in 30 of which the inoculation produced the type of organism found in the inoculated blood, while in three the result was doubtful. To these may now be added one case successfully inoculated with the æstivo-autumnal parasite by Zagari and Pace; Sacharoff's infection of himself with the æstivo-autumnal parasite taken from a leech; and two tertian, six æstivo-autumnal, and two mixed infections by tertian and æstivo-autumnal parasites reported by Elting.[19] There are thus at least 42 experiments in which the inoculation of a certain variety of parasite was followed by fever and the growth of the same parasite in the blood. Here must be mentioned also the transference of æstivo-autumnal and tertian infections, successfully accomplished by Bignami, Grassi, and Bastianelli, through the agency of mosquitoes. The invariable reappearance in the infected individual of the type of parasite contained in the injected blood is undeniably strong evidence of permanency of these malarial species. On the other hand, the inoculation experiments by no means prove that the so-called species are immutable *under all conditions.* Accordingly, the passage of the parasite through the bodies of mosquitoes and its reappearance unchanged in subjects thus inoculated by Bignami, Grassi, and Basti-

[19] *Zeitschr. f klin. Med.*, 1899, xxxvi, p. 491.

nelli, must have far higher value as evidence of the immutability of the species. Yet the same element of doubt attaches even to the latter experiments, which may merely indicate that the proper conditions for the transformation of species have not yet been furnished artificially.

A further line of evidence of the same general character is cited by the pluralists in the immutability of species demonstrated in individuals who have been kept under observation for months. It is a matter of common experience that patients who suffer relapses after long intervals sometimes extending over years, usually show the same type of parasite in the relapse as in the initial attack.

Calandruccio examined a triple quartan infection daily for months and found only quartan parasites, and in two cases crescents were found to persist in the blood for two and six months respectively, without the appearance of any other type of organism. Grassi and Feletti also found no change in the type of parasites in a case of quartan fever examined for two months, and in a case of æstivo-autumnal infection examined from October to March.

The permanency of the quartan infections might be expected, but the observations on æstivo-autumnal cases are valuable indications that æstivo-autumnal infections may, at least in some localities, persist unchanged through the winter. In this field, however, the unicists are able to offer cogent evidence in support of their claims. Antolisei (quoted by Bignami and Bastianelli) has seen patients with æstivo-autumnal infection remaining in the hospital all winter manifest tertian paroxysms with tertian parasites in the blood in the spring. They, however, regard these cases as examples of latent tertian infection.

On this same point the observations of Marchoux, during an extensive experience in Senegal, are of interest. He believes that, during the rainy season, in susceptible individuals the cycle of the malarial parasites in Senegal lasts 24 hours at the height of the season, but varies in different cases. In Europeans it tends to shorten and the fever becomes remittent or continuous. In the dry season, the cycle is longer, and the volume of the parasite increases. In many patients with a history of old seizures, who have acquired some immunity, the parasite increases in size and at length, he says, becomes identical with the mild tertian species. During the rainy season when the newly arrived Europeans are suffering from infection with the small ring forms, the native

muleteers, when attacked, all show the large tertian forms in the blood. These observations, while evidently lacking in precision, must be regarded as most significant of the underlying conditions governing the character of malarial infection in the tropics.

Ziemann's [20] experience led him to conclusions very similar to those of Marchoux, viz., that the biological and morphological peculiarities of the parasite may be altered by change of climate and differences in individual susceptibility. Thus in a patient who had recently returned from Kamerun, typical quartan fever was found associated with æstivo-autumnal parasites, while in another instance an ordinary tertian fever was developed. Ziemann also noted the prevalence of irregular fevers with small parasites among Europeans, while the natives were suffering from quartan infections. In his monograph on malaria [21] published in 1898, Ziemann opposes the unicists, and acknowledges himself as a supporter, so far as existing evidence goes, of the doctrine of plurality of species.

My observations at Montauk on cases recently arrived from Cuba, and later in New York City, developed some facts of interest in this connection.

In two cases examined in August large tertian and small æstivo-autumnal ring forms and crescents were found in the blood, but when these cases were examined three weeks later only large tertian parasites were found after a prolonged and repeated search. An irregular administration of quinine had apparently rid the blood of the pernicious type of parasite, leaving the benign tertian form to reappear in the relapse.

Of 335 cases in which parasites were identified in the blood, at Montauk, only 20 per cent (including mixed infections) showed the large tertian organism. But during the past winter (1898-99) I examined the blood of 15 volunteer soldiers who were suffering from relapses of malarial fever contracted in Cuba, and all showed the large tertian parasite only. This same experience has been the rule at various hospitals and dispensaries of this city. In one of these patients the blood was examined in August by a competent observer and found to contain æstivo-autumnal rings and crescents, but in Jan-

[20] *Centralbl. f. Bakter*, 1896. xx, p. 653.

[21] Ueber Malaria- und andere Blutparasiten.

uary only the tertian parasite could be found during a sharp relapse. It is of course possible that mixed infection existed in this case, but there is no clear explanation of the failure to find the tertian parasite in August. Also, to reconcile the fact that the tertian parasite was always found in the above-mentioned relapses with the theory of immutable species requires considerable straining of the facts known in regard to the relative virulence of the species and their relative susceptibility to quinine. It appears more reasonable to suppose that under the influence of cold weather, and gradually increasing systemic resistance, the æstivo-autumnal parasite was replaced by the tertian in the relapses suffered by these volunteers. Or it may be suggested that the cold weather alone stamped out the æstivo-autumnal infections, leaving only the tertian cases to relapse during the winter.

The disappearance of the æstivo-autumnal infections during the late autumn and winter recalls the fact that the distribution of the types of infection is determined by climatic conditions, although, as Laveran puts it, "if the species are separate, there ought to be geographical foci where tertian or æstivo-autumnal infections largely predominate, whereas all forms of malaria are commonly contracted wherever malaria is endemic."

The theory of "mixed infections" also has been made to bear a heavy burden in order to support the belief in separate species. In the ordinary type of mixed infection the tertian amœboid forms are associated with crescents, but one rarely finds both amœboid forms, with or without crescents, in the same individual. Yet if the patient is susceptible to malaria why should he retain the mild tertian amœba, while the small malignant forms disappear?

The lack of permanency observed in the mixed infections is also a suspicious feature of the condition. Two types of parasites seldom remain long together in the same subject, one very shortly displacing the other, as shown both by clinical observation and in experimental infections (cf. De Mattei, Calandruccio). As a rule, it is the more highly vegetative tertian parasite which in clinical experience displaces the more malignant æstivo-autumnal, but this rule may be reversed in experimental infections. De Mattei saw an old quartan

infection disappear after experimental infection by the æstivo-autumnal parasite, as well as the disappearance of æstivo-autumnal parasites after inoculation with quartan. Gualdi and Antolisei record two cases of quartan infection seen in May, which showed æstivo-autumnal parasites in the autumn.

The frequency of mixed infections is undoubtedly an argument against the plurality of species, showing that there is a very close connection between the sources of the tertian and æstivo-autumnal forms of parasite. In my preliminary report of the Montauk cases there were noted 12 examples of double infection out of 86 tertian cases observed. By a subsequent review of a minority of these specimens the number of mixed infections has been nearly doubled by the discovery of single crescents in tertian cases, or of single large tertian organisms among many crescents. I believe that mixed infections are much more common in the severe cases of the tropics than present reports indicate, and that their recognition depends largely on the time one cares to spend on the examination of the blood.

All the above difficulties may, however, be adjusted to the theory of a plurality of species, and in the absence of more definite knowledge of the extracorporeal form and development of the parasite it is unlikely that the question can be settled on such general considerations as those adduced.

Turning to the comparative morphology of the parasite, the evidence both for and against the plurality of species becomes much more specific. Here the pluralist doctrine finds its chief support and, whatever may be the final outcome of the discussion, it cannot be doubted that the three groups of parasites, quartan, tertian, and æstivo-autumnal, exhibit morphological characters which are to a large extent immutable. Yet the two widely different forms—the æstivo-autumnal rings and the crescents—are regarded as belonging to the same species, and the whole groundwork of a morphological classification is found to be insecure on account of the extreme polymorphism observed throughout the entire group of protozoa. Of this a few details may here be briefly considered.

One of the most striking differences between the tertian and the æstivo-autumnal parasites is the dissimilarity in the staining quality of

their nuclei. Methylene blue stains the nucleus of the young æstivo-autumnal ring densely but fails to stain the nucleus of the tertian ring. This difference probably depends upon an unequal mixture of oxy-chromatin and basi-chromatin in these nuclei. I cannot find that similar differences are recognized in the staining qualities of any two species of coccidia or of gregarines, but somewhat similar differences were noted betw ⊿ different phases of the same species of coccidia by J. Clarke, and by Siedlecki.

The study of flagellated bodies in related protozoa may be found to bear on the question of a plurality of species of malarial parasites. In *Coccidium oviforme* flagella have been found to develop from large spheroidal bodies. This protozoan produces crescents, but these have not been traced through exflagellation (Simond). In *Benedenia ovata* flagella have been found to develop from large spheroidal bodies. This protozoan also produces crescents, but these have not been traced to a flagellating stage. In *Adelea ovata*, on the other hand, the exflagellation of crescents has been observed, but large spheroidal forms producing crescents have not been described (Siedlecki).

It would appear that most coccidia and gregarines produce flagella both from large spheroidal bodies and from crescentic forms. Although the homologues of these forms in the malarial parasites are not fully determined, this fact, if fully established, would strongly indicate that the tertian malarial parasite producing flagella from large spheroidal bodies, and the æstivo-autumnal parasite, with flagellating crescents, are phases of one and the same protozoan.

It may be claimed that if the so-called malarial species are interchangeable, transitional forms ought to be abundantly present in some cases, but these have not been fully demonstrated. Yet it appears by no means certain that in order to establish the unity of the malarial parasite it is necessary to assume the existence of transitional forms. In *Benedenia octopiana*, the formation of crescents is preceded by an entire bisexual cycle of development in which the male element is furnished by large spheroidal flagellate bodies. The intermediate forms of the two cycles in this protozoan differ considerably from each other, there are no transitional forms between them, and yet both belong to the same parasite.

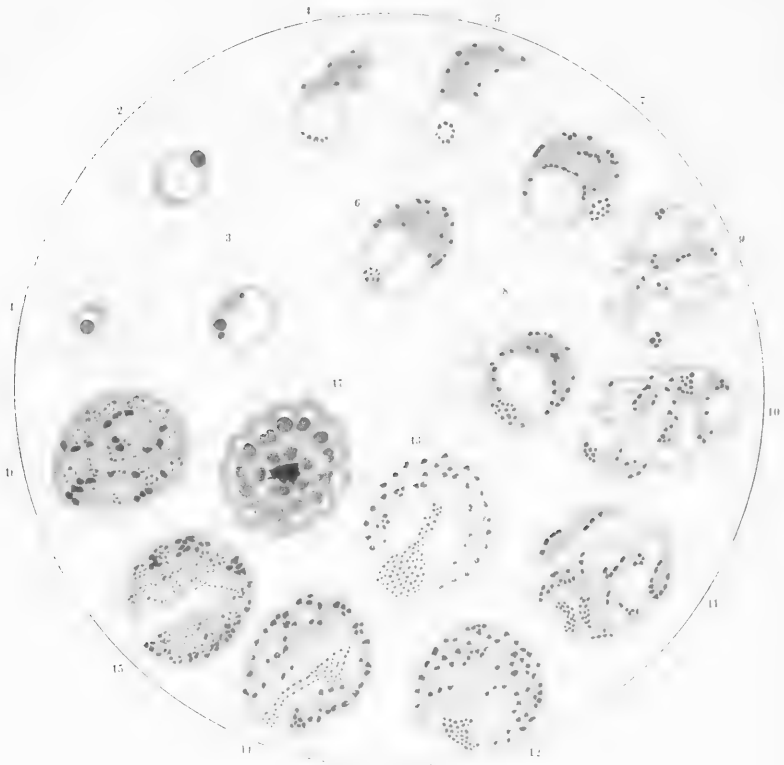
While it is true that no transitional forms between the æstivo-autumnal and the tertian parasite and between the tertian and the quartan parasites have been fully described, there are numerous observations indicating that such forms exist. The slight differences

in size, refractive quality and amœboid activity which led Marchiafava and Bignami to separate a quotidian from a tertian æstivo-autumnal species, a position from which they have largely receded, have been noticed and regarded by others as occasional differences in the morphology of one æstivo-autumnal species (Ziemann, Gautier, and others). Marchoux claims to have observed a gradual increase in the size of the æstivo-autumnal parasite during the healthier seasons in Senegal. When one closely examines the parasites seen in the average tertian case of this climate, isolated forms may be found, in greater or less numbers, which closely resemble the quartan parasite. The red cells are not always swollen when infected by tertian organisms, and these parasites are sometimes compact and very richly and coarsely pigmented at an early stage. Of the two somewhat distinct forms of parasites which I find commonly enter into the conjugating pairs of the tertian series, one is compact, of rather small size, and resembles the quartan parasite in some particulars, but the infected cells are swollen.

In examining the blood of volunteer soldiers who were suffering during the past winter from relapses of malarial fever contracted in Cuba, I was early struck with the resemblance which many of the young tertian parasites bore to the young æstivo-autumnal rings. In some of these cases the young tertian rings closely resembled the young signet-ring form of the æstivo-autumnal parasite, exhibiting a very thin bow and a distinct circumscribed swelling of one segment. Their chromatin, moreover, was often found subdivided before the appearance of pigment, although in the vast majority of mild tertian cases seen in New York City, the chromatin of the young tertian parasite is not subdivided till after pigment appears (cf. Gautier). The nuclei of these forms usually failed to stain with methylene blue, but not a few examples were found among the young rings in which the chromatin stained well with methylene blue. The usual swelling of the infected cell was often very slight, or sometimes absent, in these cases. It appeared quite possible to trace the development of these young rings up to the larger amœboid stage when the tertian characteristics become distinct. No crescents were found in any of these cases.

Several explanations may be offered to account for these peculiarities. It may be supposed that the young tertian rings do not necessarily differ from the æstivo-autumnal. This explanation I am unable to accept, finding that the tertian ring, as occurring in New York, invariably differs from the æstivo-autumnal form imported from Cuba, especially in regard to the staining quality of its nucleus (see p. 437). Or it may be supposed that the cases in question were really examples of mixed infection. Yet the suspicious young rings could be traced in development to the large tertian forms, and no crescents were found in any of these cases. Or, finally, it may be supposed that the peculiarities of the young parasites in these cases represented transitional phases between the æstivo-autumnal and tertian parasites. This explanation I am inclined to accept. The observation of 15 cases in which such peculiarities were noted is, however, insufficient to be convincing, and satisfactory grounds for the acceptance of such a belief cannot well be furnished except by demonstrating the complete transformation in the same individual of an æstivo-autumnal infection during the winter through various transitional phases in the morphology of the parasite.

Whichever theory may finally be established regarding the varieties of the human malarial parasite, the evidence would seem to justify the opinion of Kruse, Canalis, Babes, Celli, Danilewsky, and others, who regard the existence of several species as not yet proven, and who find not only in malarial parasitology, but especially from comparative biology, that the phenomena of the disease are more easily reconciled with the existence of a single polymorphous species. Certainly, in many ways, the knowledge of the disease would be furthered by adherence to the unicist theory as a practical working basis.



Developmental Cycle of Benign Tertian Parasite.

FIG. 1. Very early form of parasite, showing chromatin granule, "milky zone," and spheroidal body.

FIGS. 2, 3. Typical young ring-shaped parasites.

FIGS. 4, 5. Subdivision of chromatin, development of body and appearance of pigment in later ring-forms.

FIG. 6. Double rings, in single parasite.

FIGS. 7, 8. Turban-shaped parasites. Secondary rings, eccentric position of chromatin.

FIG. 9. Double infection of cell.

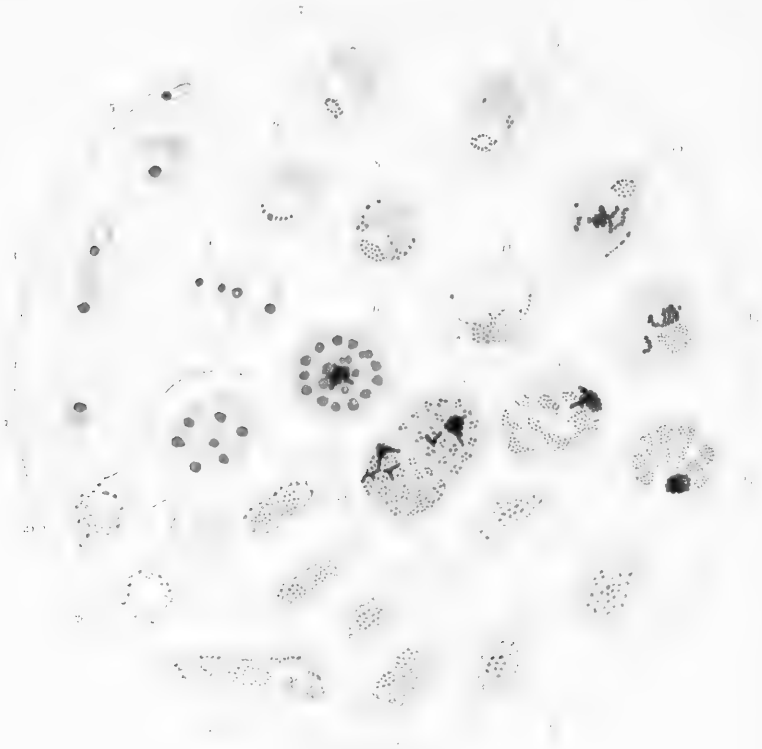
FIGS. 10, 11. Complex amœboid figures in doubly infected cells.

FIG. 12. Full grown form, with large eccentric nucleus.

FIGS. 13, 14. Protrusion of chromatin granules and milky substance in body of full-grown parasite.

FIGS. 15, 16. Division of chromatin granules into groups in reticulated presegmenting bodies.

FIG. 17. Tertian rosette.



Cycles of Æstivo-autumnal Parasite.

FIG. 1. Very young form.

FIG. 2. Infection of one cell with seven young parasites. (Drawn from a marrow-smear.)

FIG. 3. Triple infection. Two parasites joined by single chromatin mass.

FIG. 4. Double infection. Peculiar rings with two chromatin grains at opposite poles.

FIG. 5. Double infection. Small ring adherent to cell.

FIGS. 6, 7. Signet-ring forms. Sub division of chromatin.

FIGS. 8, 9. Later ring forms, with sub-divided chromatin and few pigment grains.

FIGS. 10–12. Full-grown forms with finely sub-divided chromatin and gradual concentration of pigment.

FIGS. 13, 14. Stages of presegmenting forms, with concentrated eccentric pigment.

FIG. 15. Double infection with separate presegmenting bodies.

FIG. 16. Æstivo-autumnal rosette.

FIGS. 17, 18. Young crescent and ovoid.

FIG. 19. "Pulsating" crescent.

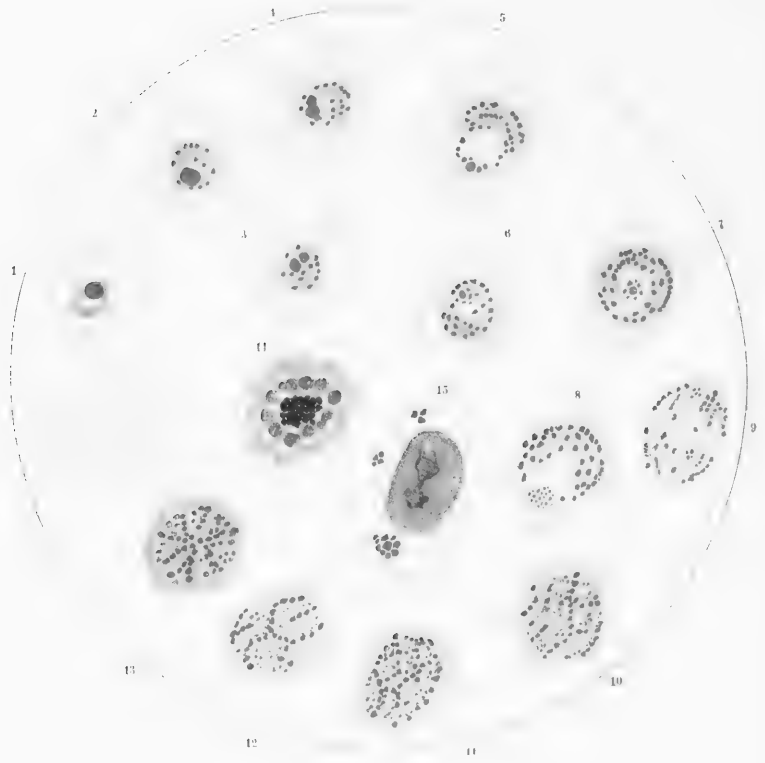
FIGS. 20–22. Various forms of crescents.

FIG. 23. Two bows about single crescent.

FIG. 24. Finely developed crescent; two masses of chromatin; achromatic substance; double wreaths of pigment.

FIG. 25. Diagrammatic flagellating body.

FIG. 26. Extra-cellular sterile body.



Cycle of Quartan Parasite.

FIG. 1. Very early non-pigmented form.

FIGS. 2, 3, 4. Small quartan rings, with large chromatin masses and abundance of pigment.

FIG. 5. Turban-shaped ring, with subdivided chromatin.

FIG. 6. Subdivision of ring and of chromatin granules.

FIG. 7. Coarse quartan ring with central chromatin granules.

FIG. 8. Full-grown quartan parasite, with eccentric chromatin, hyaline body, and abundance of pigment.

FIG. 9. Extra-cellular reticulated body.

FIGS. 10–13. Quartan presegmenting forms.

FIG. 14. Quartan rosette.

FIG. 15. Pigmented mononuclear leucocyte.

Conjugating Cycle of Tertian Malarial Parasite.

FIG. 1. Single compact body with double chromatin masses.

FIG. 2. Conjugating rings of unequal size.

FIG. 3. Double infection with a coarse ring, double chromatin granules, and a thin ring form.

FIGS. 4, 5. Early stages of conjugation of a thin ring and a compact body.

FIG. 6. Early amœboid figures of conjugating rings.

FIG. 7. Double nuclei in amœboid parasite.

FIG. 8. Union of nuclei, and subsidence of amœboid motion in older conjugating parasites.

FIGS. 9, 10. Stages of union of bodies and of three chromatin masses, of two conjugating parasites.

FIG. 11. Complete union of bodies and nuclei.

FIGS. 12, 13. Comparative sizes of full-grown forms developed with and without conjugation.

THE NERVES OF THE CAPILLARIES, WITH REMARKS ON NERVE-ENDINGS IN MUSCLE.

A NEW THEORY OF LYMPH-FORMATION AND OF GLANDULAR SECRETION.

By Chr. Sihler, Ph. D., M. D., Cleveland, Ohio.

Taking leave of the Johns Hopkins University in 1880, where I had spent three of the most satisfactory years of my life, I set myself the task to investigate the submaxillary gland in order to find the terminations of the chorda tympani. The reason for this was the importance of that nerve to physiology. To those not interested particularly in physiological questions, I may say that the chorda tympani may be looked upon as the key to solve the question of the act of glandular secretion as well as of lymph-formation in general; and as the anatomical arrangements of the submaxillary gland and its nerves are such that physiologists can undertake experiments on these nerves with very clear and lucid results, it is of course very desirable that it should be clearly understood which tissues are really influenced and made to act when the chorda is subjected to electric stimulation.

As nineteen years have now passed by since I attacked this problem, and as I have devoted all my spare time—so far as original investigation is concerned—to this and allied questions, I consider it not out of place to report my results as briefly as possible, particularly as my investigations have brought out facts which have not found a place in our anatomico-physiological conceptions, and as the views which I have been forced to adopt are by no means in harmony with the histological and physiological teaching of the present day.

Of one working on the nerve-endings of the chorda, it will very properly be expected that he make himself familiar with the endings of other motor nerves, particularly those in muscle; and this I have done in the hope of finding here histological facts which would throw some light on the nerve supply of the gland. In muscle tissue we

find three kinds of nerves: those going to the muscle spindles; the motor nerves proper ending on the muscle fibre; and the nerves going to the capillaries and other blood-vessels. The last-mentioned ones interest us directly, the motor ones indirectly, and these will be touched upon later. The former can be found by treating frog's muscle according to the acetic-acid-hæmatoxylin method which I described some years ago,[1] and which is published in Boehm and Oppel's handbook.[2]

Teasing out muscular tissue, one finds among the motor nerve fibres fine non-medullated fibres, which can be traced to the capillaries. The nerves running alongside of the capillaries can also readily be found with medium powers, but these are not the terminal branches. With the immersion lens there can be seen attached to the capillary wall some still finer nerve fibres, which pass off from the fibres just mentioned. These also have their nuclei and differ from nerves running alongside of the capillaries by their small caliber and their varicosities. They are so intimately attached that it is not easy to trace any fibre a long distance, but their nuclei always reveal them.

Although L. Bremer[3] pointed out these nerves some time ago, and although his description of them, so far as their connection with the capillary vessels and their appearance is concerned, agrees with my own observation, these nerves have not found a place in our histological reasonings, and perhaps it is just as well that they have not—at least on the strength of Bremer's work, because his other statements about them I consider erroneous. Kölliker, in his "Handbuch der Gewebelehre" (6th edition), in the paragraph on the sensory and vascular nerves of muscles, discusses these nerves in the sterno-cutaneous muscle of the frog, but adds that regarding their origin and destination he has not been able to form any definite opinion. This illustrates perhaps the state of our knowledge about them. Bremer, it is true, claims that they are branches of the motor nerves proper,

[1] *Cleveland Medical Gazette*, 1894–5, x, p. 255; and *Arch. f. Physiol.*, 1895, p. 202. The method is described also in the *Zeitschr. f. wiss. Zoologie*, 1900, lxviii, p. 323.

[2] Boehm and Oppel. Taschenbuch der mikroskopischen Technik. München, 1896.

[3] *Arch. f. mikr. Anat.*, 1882, xxi, p. 663.

but I have always found that, where I discovered such fine nerve fibres free amongst muscle fibres, I could find hidden away, amongst the motor fibres, some fine non-medullated fibrils, from which they took their origin, and I have never seen them branch off from a motor nerve, although one can readily understand how Bremer came to make this statement, so deceptive are the appearances in many instances.

These nerves, surrounding the capillaries, come from a special set or class of nerve fibres, whose appearance would lead one to consider them as belonging to the vasomotor system.

Further, in regard to their connections in the periphery, I disagree also with Bremer, who says that, aside from connection with the motor nerves, they observe a strict seclusion, never leaving the vessel. Specimens from the frog's tongue show that these nerves form connections with the sensory nerves, as the nerve fibres from the papillæ can be seen to pass into the network of nerves surrounding the vessels. This fact would lead us to think of them as sensory nerves.

Lastly, if the nerves surrounding a capillary are traced towards the centre, they can be seen to pass into the plexus surrounding the larger vessels. Very frequently two fibres can be found running on the walls of the capillaries, and if one compares the nerve supply of striped muscle with that of the capillaries it can be seen that the latter are far more richly supplied with nerves than is striped muscle.

Tracing the fibres of the chorda tympani in the submaxillary gland, I came to the conclusion that the gland cells themselves are not supplied with nerve fibres, but that the terminal fibres are found on the capillary vessels, just as in the case of the capillaries of muscular tissue, and that, therefore, those nerves of muscle which are analogous to the glandular nerves are not the motor nerves proper, but are those going to the capillaries.

The important fact which I wish to point out in this connection is that, inasmuch as the chorda is considered universally as a motor nerve, and if I am right in placing the nerve terminations of the chorda on the capillaries, it would be established that the capillary nerves are fed by motor trunks and that their function is of a motor character.

To sum up the histological facts, then, there exists a vast peripheral network of fine nerves coëxtensive with the capillaries of the muscles and glands, which has connection with sensory nerves and into which motor-nerve trunks also enter, and which I therefore look upon as being sensory and motor at the same time. Whether there is a distinction here between sensory and motor fibres, or whether any single fibre may be both, does not concern us. I have no difficulty in imagining the latter to be the case, as every neurone, whether it be called sensory or motor, both receives and imparts impressions. We ought not without proof to apply conceptions pertaining to the well-selected strands of fibres in the spinal cord to a peripheral plexus where direction has little to say. The existence of such a plexus would not support the hypothesis that the entire nervous system is made up of independent neurones.

What is the significance of these nerves of the capillaries, what is their function? Although I am not so fortunate as to have at my command a laboratory in which I could experiment on these nerves, yet if we take into consideration the histological facts together with certain clinical observations and physiological experiments which have been made on these nerves, although without any accurate anatomical knowledge about them, we have, I think, satisfactory evidence and scientific support for the hypothesis which I shall here state briefly: These nerves, so intimately connected with the capillaries, influence the protoplasm of their walls in such a way that, according to the activity of the nerves, the transudation of lymph is increased or diminished. Further, they take cognizance of local disturbances of a chemical or mechanical nature, and in response to local causes of irritation influence the capillaries of a part to pour out more fluid and act in the interest of the organ in question. As increase of lymph-formation and vasodilatation must, in the long run, go hand in hand, it would seem reasonable to suppose that the nerve fibres going from the capillaries to the arteries and veins may exert an inhibitory influence on the vasoconstrictors, or a stimulating one on the vasodilators, whereby a larger supply of blood is furnished to the irritated part.

I will now enumerate the reasons which give support to the hypothesis mentioned. I must premise, however, by way of explanation, that when I speak of increased lymph-production by the capillaries, I consider that this is brought about not by simple increase in the caliber of the vessel, but by some specific action of the cells of the capillary wall—an activity as specific for these cells as contraction is for muscular tissue.

(1) The first reason offered in support of my hypothesis is based upon our knowledge of the physiological properties of the capillaries as manifested in processes of transudation. Michael Foster in discussing the problem of lymph-formation says that "the condition of the vascular wall so profoundly influences the transit of material as to render the process very complex. We may probably regard it as too complex to be compared even with filtration through a filter capable of widely changing in texture from time to time and as more nearly resembling the process of secretion." My views are in exact accordance with these words of this distinguished physiologist.

In the familiar facts of muscular contraction we have unmistakable evidence that it is possible for living tissue to experience the most profound molecular changes through the influence of nervous action. Why should this not be possible for other tissues? That very marked changes can take place in the capillary wall is evident from a consideration of what occurs during glandular activity and during the process of inflammation.

However glandular secretion may be brought about, it would seem impossible that the capillary wall should be wholly indifferent while the saliva and other secretions are poured out from the ducts so promptly and abundantly. There is sufficient physiological, as well as pathological, evidence to indicate that we cannot look upon the capillary wall as containing physical pores open to suction from the gland cells and to the direct action of the blood-pressure. The vital condition of the capillary wall must be different when secretion is going on from that when this is not the case.

Further, when inflammation exists, not only are larger quantities of lymph exuded through the walls of the capillaries, but even cor-

puscular elements find their way through these structures; and yet after the pathological process has run its course, the capillaries return to their original state, thus illustrating what great changes can take place in the cells of the walls of the smallest blood-vessels.

(2) A second support of the hypothesis is to be found in the histological facts mentioned—the enormously rich supply of nerves to the capillaries. These must have their purpose, and, if my view that the chorda tympani ends on the capillaries should be confirmed, scarcely any further proof of the hypothesis would be required. Although my observations have been limited to muscular tissue and the salivary glands, these two tissues are sufficiently representative to make it probable that the capillaries in other organs are supplied with nerves of a like character.

(3) When the chorda tympani is stimulated the saliva appears in Wharton's duct under a pressure which may be greater than that in the carotid artery; atropia stops the secretion even if, through nerve stimulation, the vessels remain dilated; and even after cutting off the blood-supply, saliva is said to be secreted.

To explain these well-known facts, simple vasodilatation being insufficient, physiologists have called into service the gland cells themselves and have ascribed to them nerves which are supposed to incite the gland cells to secretion just as stimulation of the motor nerves causes muscular contraction. The internal changes, however, in muscle during contraction are widely different from those in active glands, whose secretion is composed mostly of water.

Let us analyze this assumption of the physiologists. Let us assume that the gland cells are provided with nerves and that some internal changes go on in these cells in consequence of their stimulation. How can these changes influence the capillary wall, as no nerves pass from the gland cell to this wall, and as in the capillary there are no open pores through which a sucking action could be carried on? Upon this assumption I see no other way by which the capillary could be influenced to produce more lymph than by supposing that it is sensitive to the state of concentration of its surrounding lymph, and that greater concentration of this will induce the capillary to pour out more fluid.

To my mind the gland cells are not constructed to furnish large quantities of fluid, they are not in immediate contact with the source of supply, while the capillary cell, with its flat surface and thin walls, continually bathed with serum on its inner surface, would seem to be made for such a purpose. I cannot understand why gland cells should have their own nerves, while an independent action of these cells manifested in the formation of chemical substances, without the production of fluid to wash away and dissolve them, would be of no use to the organism. On the contrary, I can see that, if provision were made to have large quantities of lymph poured out over the gland cells, that this influence would act as a sufficient stimulation to the gland cell to transform some of its reserve material into soluble matter and to push on the fluid received from the capillaries. As the water in glandular secretions is to be accounted for as well as the dissolved substances, as a separate nerve supply to the gland cells seems to be physiologically unnecessary, and as I have satisfied myself of the existence, both in muscular tissue and in the salivary glands, of a rich supply of nerves to the capillaries, I cannot without better evidence than we now possess admit the reasoning of the physiologists on this subject as sound.

(4) The fourth point relates to the results of experiments on the branch of the chorda going to the tongue. The chorda tympani coming from the skull divides into two parts, one going to the submaxillary gland, the other to the tongue.

In Cohnheim's "Pathology,"[4] Ostroumoff's experiment is mentioned and discussed, and as I consider it important and interesting, I shall introduce it here:

"If," Cohnheim says, "the peripheral stump of the dog's cut lingual nerve is stimulated for a period of time by induction currents of increasing strength, the rapidly forming intense hyperæmia of the corresponding half of the tongue is accompanied by a pronounced œdema, which, in about ten minutes after the beginning of the stimulation, is evident to the naked eye, and in the next ten minutes increases uninterruptedly to a very considerable size. This very remarkable experiment,

[4] Vorlesungen über allgem. Pathologie, Bd. i, p. 134. Berlin, 1882.

about which I have been kindly informed by M. Ostroumoff, is indeed calculated to raise doubts whether all congestions, however brought about, are of equal significance so far as lymph-formation is concerned, and the more so as there are certain observations on man, for example, the rapid formation of urticaria wheals, due to undoubted nervous influence, which point only too clearly to the *existence of intimate relations between the formation of lymph and the innervation of vessels.*" (The italics are my own.)

We have evidence, then, that stimulation of the branch of the chorda going to the submaxillary gland results in the production of fluid contained in Wharton's duct and that stimulation of the branch of the chorda going to the tongue likewise produces fluid, which is here contained in the lymph spaces of the tongue. Now it might be expected that the formation of lymph by the different branches of the same nerve, called forth by the same agency, would be explained on similar physiological principles as brought about by similar histological elements. But no; in the case of the gland, while the vessels are allowed to supply the organ with more blood, the gland cells are summoned in order to explain the occurrence of the fluid in the duct; while in the case of the tongue an ill-defined vasomotor action has to account for the accumulation of fluid in the lymph spaces.

If it is considered possible that, in consequence of the stimulation of gland cells by *hypothetical* nerves, these cells can not only form mucus, but produce from somewhere large quantities of fluids, why, I would ask, may it not be assumed that nerves that *can be demonstrated* can incite the cells of the capillary walls to increased transudation of lymph, which passing to the gland cells will induce them to add their chemical product to it, while retaining the albuminous matter, and to pass both fluid and product on into the ducts. This hypothesis demands no new conceptions, but merely a transference of accepted physiological doctrines from the gland cells and their problematical nerves to the cells of the capillaries and their demonstrated nerves. Ostroumoff's experiment on the chorda branch going to the tongue demonstrates plainly that the increased flow of fluid from the vessels into the tissues can be accounted for without assuming the participation of gland cells.

(5) Further support for the views here advocated can be found in experiments made by Rogowicz[5] under Heidenhain's direction to clear up the connection between vasodilatation and lymph-formation. He shows by actual measurements that all forms of dilatation are followed by an increased flow of lymph. This fact does not interest us here particularly, as this increase might be explained simply by the larger blood-supply to the part. Another experiment, however, which he instituted in order to get an insight into the promptness with which an increase in lymph-transudation is brought about by stimulation of the chorda of the tongue, is of more interest to us. "While," says Rogowicz, "the lingualis was being stimulated, a saturated solution of sulph-indigo-carmine was injected into the saphenous vein. The aspect of the tongue under these circumstances is most remarkable, while the circulation of the blood on the side of the tongue not stimulated is yet in a normal condition. The half of the tongue corresponding to the stimulated lingual nerve takes on a deep blue color in a very short time, and that, too, at a point of time when the other side presents only a pale blue hue. If the injection is now discontinued this difference in coloring will continue for quite a while. It depends on a more rapid filtration of lymph into the tissues of the stimulated half of the tongue." Rogowicz then explains that the blue color is not due to the coloration of the blood, which remains red, but that the coloring matter is in the connective-tissue spaces throughout the tongue, as can be seen in transverse section through the hardened tissue. This very prompt and energetic lymph-formation could hardly be explained by simple dilatation of the arteries.

Of further importance to us here are other facts brought out in the course of these experiments. Rogowicz found that after injection of curare the production of lymph may be increased three to four times, without any apparent increase of the blood-supply, as, for example, from 35 cc. to 115 cc., and from 81 cc. to 240 cc. We thus see that with a stationary blood-supply lymph-formation can be increased. On the other hand, the experiments on the submaxillary gland have shown that atropia will prevent the formation of lymph even if vasodilatation is brought about by nervous influence.

[5] Pflüger's *Archiv*, 1885, xxxvi, p. 252.

(6) Heidenhain[6] in his researches on the pseudomotor contraction of the tongue has made some observations which speak strongly in favor of the view defended in this paper. Searching for the cause of the contraction of the tongue, the muscle fibres of which had been made hyperæsthetic through section of the hypoglossal nerve some days previously, he found that stimulation of the chorda, which under these conditions causes contraction of the lingual muscles, was always followed by hyperæmia of the tongue. Heidenhain's surmise that the immediate stimulus to the muscle contraction is an increased flow of lymph was supported by the subsequent experiments of Rogowicz made under his direction. Under the conditions here present even physiological salt solution, towards which normal muscle is indifferent, will produce active muscular contractions.

The two facts, however, which are of most importance to us are that, when Heidenhain had clamped the arteries supplying the tongue, stimulation of the chorda still produced contractions, though of diminished force, as might be expected, and only for a short time; and that these contractions would take place upon stimulation of the chorda before any visible hyperæmia, indicating a pouring out of lymph from the capillaries, had set in. Heidenhain says in closing the discussion:

"After these observations it does not seem improbable that a rapid increase in the amount of fluid saturating the tongue may be looked upon as the cause of the contractions of the paralyzed muscle. But I must lay particular stress on the point, that I have not offered convincing proof for this assumption. . . . Above all, the evidence is lacking that even after interruption of the circulation in the tongue stimulation of the chorda can produce a transudation of lymph."

Regarding this last statement, it seems to me that the occurrence of muscular contractions due to stimulation of the chorda furnishes the evidence for lymph-formation through action of the capillaries themselves. When the arteries are clamped, the chorda can influence nothing else but the capillaries, and as it has been shown, on the one

[6] *Arch. f. Physiologie*, Suppl.-Bd., 1883, p. 133, and Rogowicz, Pflüger's *Archiv*, 1885, xxxvi, p. 1.

hand, that lymph-formation can be induced without increase of blood-supply; and, on the other hand, that these degenerating muscles of the tongue respond even to such feeble stimulation as injections of normal salt, it seems justifiable to regard these contractions as an evidence for lymph-transudation brought about by activity of the capillaries.

At any rate, the proof for this function of the capillaries is a good deal stronger than that now considered sufficient to explain the production of the watery parts of the saliva by action of the gland cells and their hypothetical nerves. When the high pressure in Wharton's duct, the action of atropia, the secretion of saliva after severance of the head, are adduced to demonstrate the activity of the gland cells, it must not be forgotten that not only the gland cells and their hypothetical nerves, but also the capillaries and their real nerves, should be taken into account as factors in the phenomena. In the case, however, of these contractions of degenerating muscles in the tongue, with arteries clamped and chorda stimulated, we have to do solely with the capillaries and their nerves. We can exclude everything else. No one has ever separated under analogous circumstances the gland cells in order to see what function they can then perform.

(7) Attention may be called also to injuries of the cornea by a foreign body or other irritant, as throwing light on the character of our nerves. When the fundamental experiment of Cohnheim is carried out on the mesentery and the changes in the capillary walls are observed, these are at present usually explained by the injury directly inflicted on the capillaries. But when a piece of steel in the centre of the cornea sets up not only a violent injection of the conjunctival vessels, but also an increased flow of lymph and an emigration of white corpuscles, we cannot explain these phenomena by a direct injury to the vessels. If, as generally believed, a change in the walls of the capillaries is necessary to explain the emigration of white blood-corpuscles in inflammation, then we have here an illustration of the influence of sensory nerves upon the nerves of the capillaries, and not solely such an influence upon those of the arteries and veins.

(8) Heidenhain, who is the main authority for the prevailing

theory of secretion, which imposes upon the gland cells the function of drawing the necessary amount of fluid from the blood, and passing it into the duct, points out an apparently very serious objection to this theory in the œdema which takes place when, the ducts of the submaxillary being tied, the chorda is stimulated. He says: [7]

"This phenomenon seemed to be irreconcilable with any form of attraction-hypothesis, and to be in favor of a propelling force for the stream of fluid, a force independent of the gland cells and set into action by nervous influence. But there is one explanation which had previously escaped me in spite of all my efforts to find one. As soon as the œdema begins to develop the tension of the connective-tissue capsule surrounding the gland increases to a high degree, which must have the effect of compressing the veins which pass through the capsule. Thus the artificial filtration-œdema causes, through the difficulty it establishes for the escape of blood from the gland, an œdema due to passive venous congestion (Stauungsödem), which becomes greater as the stimulation of the chorda increases the blood-pressure in the capillaries."

As bearing upon Heidenhain's explanation, the following questions seem pertinent:

(*a*) How is that part of the œdema explained which sets up such a very marked tension in the capsule that it shuts off the circulation? Would not this interfere with the whole process of secretion when little or no fresh blood can pass through the gland?

(*b*) What evidence is there for such constriction of the veins?

(*c*) Would not the gland cells, as soon as they found an impediment to the passing on of the water which they had taken out of the blood current, cease their activity, so that such an œdema would not take place?

(*d*) Would it not be more satisfactory to explain this œdema in the gland in the same way as Cohnheim and Ostroumoff explained the œdema of the tongue after stimulation of the chorda branch, where there is no impediment to the outflow of blood through the veins?

Heidenhain and other physiologists have, so far as I am aware, never considered nor mentioned the theory which we propose, and I think for several reasons:

[7] Hermann's Handb. d. Physiologie, Bd. v, Theil 1, p. 77. Leipzig, 1883.

First. Physiologists have been so impressed with the analogy of glandular secretion to muscular contraction that they have assumed without sufficient evidence that motor nerves of glands exist and excite the gland cells to activity.

Second. It has not heretofore been established that the capillaries are richly supplied with nerves and that these are of a motor character.

Third. In speaking of vasomotor activity, the functions of the larger vessels and those of the capillaries have not been clearly distinguished from each other.

While the facts and arguments which I have thus far considered seem to me to speak strongly for an independent activity of the capillaries under the control of the nervous system, the decisive point to be established is whether the chorda terminates in the submaxillary gland on the capillaries and not in the gland cells. The establishment of this point would outweigh in importance all of the other considerations adduced, as the chorda tympani is acknowledged to possess motor characters. It is, therefore, important to give shortly some reasons why I believe that the chorda tympani terminates on the capillaries.

In this connection, as will appear, a true knowledge of the motor nerve endings in muscle is of importance. The present teaching about motor endings in muscle is that the end fibrils penetrate the sarcolemma and as naked end-fibrils come into contact with the muscle substance. My investigations have led me to a different conclusion. I find that the motor endings remain on the outside of the sarcolemma, and, except at the surfaces, where muscle and nerve come into contact, are covered with the strong sheath of Schwann which has its own nuclei. What may be the exact condition of things at the points where muscle and nerve fibre are in actual contact—whether the sarcolemma and neurilemma are wanting there, or perforations exist, or whether electrical phenomena observed in nervous activity can be used to explain the processes going on there—I cannot say. The precise relation of muscle to nerve here is an unsolved and difficult histological problem.

It would lead too far to present here in full the evidence in support

of my views concerning these nerve-endings. Much of it I have brought forward in other papers,[8] but a few points may be here adduced. The important fact is that these motor endings have their own nuclei. Formerly these nuclei gave no trouble to the advocates of the so-called hypolemmal theory, as one could consider them as belonging to the axis cylinder. But now when we know that the axis cylinder is a process of a cell in the brain, spinal cord or one of the ganglia, and that this process possesses no nuclei of its own, we must ascribe the nuclei in the nerve-endings in muscle to the sheath of Schwann, and why should we not? They agree in appearance with the nuclei of other fine nerves, where there is no question but that the nuclei belong to the sheath of Schwann. But if this sheath is continued over the end fibrils, its presence would prevent direct contact between nerve substance and muscle, even if the endings were placed beneath the sarcolemma.

Lately, it is true, Huber and De Witt[9] have announced that these end fibrils are devoid of nuclei, although Kühne and Kölliker have described them. The advocates of the hypolemmal theory should rejoice at this discovery, inasmuch as the presence of these nuclei would deal the death-blow to that theory. I cannot here enter into a criticism of this observation of Huber and De Witt, and will only say that I have seen these nuclei attached to the end fibrils in hundreds of perfect specimens and that I consider the evidence advanced by these investigators as inconclusive and their methods as inadequate to settle this point.

Further evidence for the epilemmal position of the end fibrils is furnished by a class of nerve endings in the frog's muscle, not described in the text-books. Here we find not only the form described in the text-books, but also a variety of other endings. There are terminal branches of non-medullated nerve fibres, which, after attaching themselves to a muscular fibre for a little distance, form an arch quite free from the fibre, again come into contact with the muscle and then, perhaps, form a second arch, and so on. The nuclei may be found

[8] *Arch. f. mikr. Anat.*, 1895, xlvi, p. 709; 1900, lvi, p. 334, and *Zeitschr. f. wiss. Zoologie*, 1900, lxviii, p. 323.

[9] *Journal of Comparative Neurology*, 1897–8, vii, p. 169

as well on the free arch as on the attached portion of the nerve fibre. There are other forms where these arches grow smaller, but the festooned arrangement can still be recognized. Now these arches, whether large or small, show clearly where the end fibre is situated, for it is impossible to place them under the sarcolemma, and it would be an unwarrantable assumption to suppose that in one case the nuclei and end fibrils are above the sarcolemma, and in the other under it. The forms with small arches have been misunderstood both by Kühne and by Bremer. Kühne described them as artefacts due to the gold method, and at the same time inconsistently spoke of them as a special class of atypical endings occurring more frequently in certain parts of the body than elsewhere.

The hypolemmal theory was announced many years ago when the methods were very imperfect, although they yielded valuable results. I would here point out that a figure in Stricker's Handbook,[10] which has been copied into many text-books, is erroneously interpreted. The nuclei which are ascribed to the sheath of Schwann are really those of Henle's sheath, so that according to this figure Henle's sheath is made to pass with the terminal fibres through the sarcolemma. If the methods of that day failed to distinguish such an important point as this, is it likely that they sufficed to determine such a delicate and subtle matter as the precise relation of the end fibrils to the sarcolemma? It is true that Kühne says that in profile views the passing of the sheath of Schwann into the sarcolemma can be observed without difficulty, but I have examined hundreds of good specimens and have never been able to convince myself of this, while, on the contrary, I have seen many facts inconsistent with this view. I have shown in recent papers[11] that in the frog and in the small muscle fibres of the snake Henle's sheath is open at the end, thus allowing the cerebrospinal fluid to escape, and that in other animals this sheath extends over the end fibres and assists in forming Kühne's "Sohlensubstanz."

These ivy-like forms of nerve-ending in the frog further show

[10] Stricker's Handb. d. Lehre von. d. Geweben, Bd. i, p. 154, Fig. 35. Leipzig, 1871.

[11] *Arch. f. mikr. Anat.*, 1900, lvi, p. 350, and *Zeitschr. f. wiss. Zoologie*, 1900, lxviii, p. 323.

that nerve fibres may influence muscle not only at the places where they actually terminate, but also at places where they merely come into contact with the muscle, the fibre itself passing on to a second muscle fibre and so on. This view of the motor nerve-endings in striped muscle is of general importance, as it indicates that in other situations cells may be influenced by similar contact with nerves without the presence of naked end fibrils. I therefore consider the plexus of fine nerves found in smooth muscle and built like certain motor endings in striped muscle, as the "nerve-endings" of this class of muscle, and consider it futile to look for a nerve-ending for each plain muscle-fibre cell.

To return to the submaxillary gland, I consider that there are sufficient analogies to justify my interpretation of the nerves which I have found there in connection with the capillaries, as the terminations in this organ of the chorda tympani, for there is agreement in histological details between these nerves and nerve terminations in plain and striped muscle, as well as the nerves of the capillaries in muscle. If one stains with Beale's carmine a small but sufficiently large piece of the gland to contain all the structures, and presses it out carefully, among the various nuclei four kinds can be distinguished: those of the gland cells, those of endothelial cells of the capsule, those belonging to the capillaries, and other nuclei just outside of the capillaries; these last are the nuclei of the nerve fibres clinging to the capillary wall. While I am able to demonstrate nerves going to the capillaries, I have failed to find nerves going to the gland cells. I am aware that Dogiel,[12] Huber [13] and others have reported finding nerve endings on the cells of certain glands, as the lachrymal and submaxillary. The correct interpretation, however, of the pictures presented in specimens treated by the methods employed by these observers seems to me to be still doubtful, and in view of my negative results on this point I am not prepared to accept their conclusions.

Since my first publication, over fifteen years ago, on the structure and nerves of the submaxillary gland, and of muscle,[14] the conclusions

[12] *Arch. f. mikr. Anat.*, 1893, xiii, p. 632.

[13] *Journal of Experimental Medicine*, 1896, i, p. 281.

[14] Studies from the Biological Laboratory of the Johns Hopkins University, 1884, iii, p. 155. *Cleveland Medical Gazette*, 1885–6, i, p. 193.

then formed have been confirmed by the application of my new method which shows that the nerve supply of the capillaries is rich enough to be looked upon as the termination of the chorda. Still further confirmation of my views has been afforded by study of the capillaries of muscle. At that time I advanced the theory of the independent activity of the capillary wall and its importance in the production of lymph, and suggested that these real nerves explain all the experimental facts fully as well as do the hypothetical ones on the gland cells.

Although I have never seen the theory which I propose in this paper offered before, it seems to have been foreshadowed by Cohnheim, as will appear from the following extracts from his "Pathology." I have italicized certain passages to which I wish to call special attention. Discussing the theory of inflammation, Cohnheim[15] says:

"But if it is impossible at the present day to give a more accurate definition of this alteration [that in the walls of the blood-vessels during inflammation] no blame can be attached to us, the pathologists, on account of the obscurity in which even the normal process of transudation is at present involved. Nobody can assign the cause for the different chemical composition of the transudates in the different regions of the body; nobody knows with certainty on what condition it depends, that the vessels of the pleura and pericardium secrete a transudate which is so much more concentrated than that of the vessels which furnish the cerebrospinal fluid. Every day some new, astonishing evidence presents itself showing, on the one hand, what an important influence the walls of the blood-vessels exert on the vital functions and, on the other, how energetically they react to all sorts of injuries. That through their activity the caliber of the vessels can be increased or diminished is a well-known fact, and has been taken into account both by physiologists and pathologists. But since it has been shown in Ludwig's laboratory that curarized animals produce lymph of higher concentration than the normal, *does not this fact point to relations of a quite different character between the vascular walls and the blood-current?* If the skin of a dog assumes a red color after an injection of curare into a vein, we are not very much astonished, as we are accustomed to ascribe to curare a paralyzing or a stimulating influence on the vascular nerves. Through this

[15] Op. cit., i, p. 282.

well-known action, curare cannot, however, exert an influence on transudation; but why should it not be able to act on other constituents of the vascular wall than solely the nerves of the muscle-fibres? Why not directly on those that are instrumental in the process of transudation, even *if these are as yet unknown to us? It is possible that nerves come into play here or that nerves can exert at least an important influence on the process of transudation,* as has been long established for a number of secretions and recently also for the blood-vessels of the tongue by Ostroumoff's experiment. Further striking evidence for the view that *the nervous system can influence the qualities of the vascular walls in a quite different way than by simply acting on their contractility* is furnished by the rapid onset of œdema in the paralyzed extremities, repeatedly observed in cases of acute myelitis, and also by Gergens' demonstration that the blood-vessels of frogs whose spinal cord has been destroyed, allow much larger quantities of fluids and even pigment granules to pass through their walls than is the case with frogs with normal medulla. Who would venture to decide in the present day whether or not such *specific nervous arrangements situated in the vascular walls themselves* come into play also in the process of inflammation?"

It is evident from this quotation that Cohnheim recognized the inadequacy of limiting the influence of nerves upon blood-vessels merely to an effect upon the caliber of the vessels, and that he brought forward strong arguments for the view that the nerves must be capable of exerting also other influences upon the vascular walls, especially in the production of lymph and in inflammatory processes. I have, therefore, great satisfaction in having pointed out, and, I hope, correctly described those nerves which Cohnheim saw with his mental vision, and I think I may say, without assuming too much, that, rightly interpreted, there is here entire harmony between physiological theory and anatomical facts.

It may have occurred to the reader that in these nerves we have at last found the nerves of nutrition, which have always very properly been bowed out of the front door by the theoretical men and just as often been called in at the back door by the practical men, but I cannot on this occasion follow up this theme farther.

I regret that in the absence of the requisite laboratory facilities I have been unable to perform certain physiological experiments bear-

ing on the questions discussed. I should especially like to repeat the experiments on the chorda of the tongue of dogs anæsthetized by water pressure on the brain by Kemp's method, so that no drugs would have touched the peripheral nerves. I would then try the action of atropia on the nerves of the tongue and see if the formation of the œdema could be thereby prevented, as is the secretion by its action on the nerves of the submaxillary gland. If that should be the case we would have further proof of the correctness of our theory.

I need hardly say in conclusion that I am well aware of the conflict between the views here presented and the current teaching in text-books, as well as the results reached by many eminent investigators. I am glad, however, to find my conclusions so largely in accord with the opinions of Kölliker and of Cohnheim.

In justice to myself, I wish to say that I am still open to conviction regarding the existence of nerves going to the gland cells. But regarding the formation of lymph and the regulation of its flow, it seems to me that the histological and physiological conditions for this function must exist, and be about the same, all over the body.

CONCLUSIONS.

1. The endings of the motor nerves in striped muscle remain on the outside of the sarcolemma. Aside from the surfaces of contact of muscle and nerve fibre, the end fibres are covered down to their tips with the sheath of Schwann and are provided with nuclei. The precise condition of things at the places of contact of muscle and nerve is an unsolved problem of histology.

2. The ivy-like or festooned arrangement of motor nerves in the frog's muscle has been misinterpreted. Properly interpreted it demonstrates that the nerve fibres that are to influence the muscle fibre are not naked and that they need not be end fibres. It shows that mere contact between muscle fibre and nerve fibre is all that is necessary.

3. The sheath of Henle in the frog and in the smaller muscle fibres of the snake is open, thus permitting escape of the cerebrospinal fluid.

4. In other animals Henle's sheath extends over the end fibres of the

motor nerve and the cells lining it envelop the end fibrils. I find that the so-called "Sohlensubstanz" of Kühne is derived from the cells of Henle's sheath.

5. The terminal nerves in smooth muscle form a network entwining the bundles of muscle fibres. I consider it improbable that each plain muscle fibre has a special terminal nerve fibril.

6. In muscular tissue fine non-medullated nerves, probably belonging to the centrifugal, vasomotor system, proceed from the fasciculi of motor nerves. These nerves can be traced directly to a network of nerves surrounding the capillaries. From this network fine, nucleated, nerve fibres pass to the walls of the capillaries, with which they are very closely united.

7. The nerves supplying the capillaries connect also with sensory nerves and with nerves surrounding the larger blood-vessels, both arteries and veins.

8. The branches of the chorda tympani in the submaxillary gland do not pass to the gland cells, but they terminate on the capillaries.

9. In muscular and glandular tissues—and perhaps throughout the body—there is a vast peripheral nervous plexus belonging to the capillary blood-vessels. These nerves of the capillaries, which may perhaps be regarded as nutritive nerves, regulate the production and transudation of lymph, and are concerned in the mechanism of glandular secretion. They may be called into activity both by peripheral influences and by impulses received from the central nervous system and the sympathetic ganglia. They may influence, through their connections with the vasomotor nerves on the arteries and veins, the blood supply to a part.

THE INFLUENCE OF BILE ON METABOLISM.*

By ELLIOTT P. JOSLIN.

(From the Laboratory of Experimental Pharmacology and Therapeutics of the Harvard Medical School and the Chemical Laboratory of the Massachusetts General Hospital.)

The influence of the bile on metabolism has been studied heretofore by observing the changes which have occurred when the bile was excluded from the intestinal tract. This paper deals with the results obtained by the administration of bile by the mouth. The incentive to this work came from my friend Dr. Franz Pfaff, to whose unvarying interest and encouragement this paper is chiefly due. In an article by Pfaff and Balch,[1] entitled "An Experimental Investigation of Some of the Conditions Influencing the Secretion and Composition of Human Bile," it was shown that human bile, ox bile and bile salts, when given to a patient with complete biliary fistula, had a marked cholagogic action. Their experiment extended over a period of ninety-seven days, and represents the most complete study of the human biliary secretion which has yet been made. During this investigation it was observed that while the patient was taking bile in one form or another the appetite improved, the bowels moved without medication, and the stools diminished in bulk, but increased in consistency and color.

The article above mentioned closes with the following paragraph: "For the present we will only say that bile may be useful in those cases where so-called cholagogues are now prescribed, as well as in certain cases of constipation, and possibly in cases where we wish to increase the absorption of fat."

The subject[2] of my experiment was a married woman, fifty-four

* A report of work done under the provisions of the second Dalton Scholarship at the Massachusetts General Hospital for the years 1898–1899.

[1] *The Journal of Experimental Medicine*, 1897, ii, p. 49.

[2] The patient was in the services of Dr. H. H. A. Beach and Dr. Maurice H. Richardson at the Massachusetts General Hospital, and the operation was performed by Dr. S. J. Mixter. To all these gentlemen I am greatly indebted for the privilege of conducting these investigations.

years of age, who entered the Massachusetts General Hospital September 26, 1898. Her family and personal history were good and she was the mother of twelve healthy children. During the last three years she had suffered from attacks of gall-stones, and on this account came to the hospital for operation. This was performed on September 29. The patient's condition became so critical while upon the operating-table that the gall-bladder alone was emptied of stones and a biliary fistula made, the duct being left untouched.

After the operation the discharge of bile from the wound was constant, the stools remained colorless, and repeated tests failed to show the presence in them of bile acids. It was during the convalescence between December 20 and 31 that the following investigations were made.

The experiment was divided into three periods, similar in all respects save that in the middle period the patient received thirty grammes of dried ox bile. The beginning and the end of the experiment, as well as the different periods, were marked off by the patient's taking the charcoal mixture recommended by F. Müller.[3] In each instance the change in the period was definitely shown by the dark color of the stools. The bowels showed no tendency at any time to constipation or diarrhœa. During these twelve days the patient passed about two-thirds of the twenty-four hours in bed, and for the remainder of the day was up and about her room.

All of the food which the patient ate was prepared and weighed in the laboratory. The diet consisted essentially of bread and butter, thin cream, eggs, sugar and beef. The percentage of nitrogen and of fat was determined in each article of food, double analyses being made daily in the case of the cream.

The urine was collected daily and the amount and specific gravity noted. The nitrogen in 5 cc. from the twenty-four hours' amount was determined by the Kjeldahl method, and the urea with Squibb's apparatus, control analyses being made in both cases. The stools for each period, with the wash-water made use of in cleaning the utensils, were made slightly acid with a few drops of concentrated sulphuric

[3] Untersuchungen über Icterus. *Zeitschr. f. klin. Medicin*, 1887, xii, p. 45.

acid, and were then evaporated over the water-bath, special care being taken to stir them thoroughly and frequently. The stools did not become solid while over the water-bath, but on removal from the same assumed a firm consistency, though not sufficiently so to allow of reduction to a powder. They were then weighed, and the total percentage of fat determined by double analyses.

The fat was first extracted with ether alone. The residue was then stirred in a porcelain dish with an 8 per cent alcoholic solution of hydrochloric acid and the mixture slowly evaporated to dryness. This residue was then ground up with sand, placed in a fat-extraction thimble and extracted again. It was found simpler to extract the fat in two stages owing to the large amount of fat present in the stools.

The bile was collected at six-hour intervals and the amount, specific gravity and the percentage of solids in the twenty-four hours' amount determined. The fistula caused much annoyance, as it did not admit of the satisfactory use of any cannula. The house officer, Dr. LeCompte, tried many devices, but it was impossible to prevent leakage. On this account that portion of the bile which was not collected in the flask worn by the patient was caught in the dressings, which were arranged for this purpose, and was subsequently extracted from them with water. This watery extract was then filtered, and the filtrate evaporated to constant weight. As the percentage of solids in the bile each day had been calculated, the amount of bile absorbed in the dressings was easily estimated. That the method was sufficiently accurate is shown by a comparison of the total amount of bile in the first and third periods. In these the amounts of bile are seen to be nearly the same, in spite of the fact that in the first period there was no leakage from the cannula, while in the third period only half the total quantity was collected through the cannula, the remainder being caught in the dressings.

Bile was given to the patient in the second period in the form of bile pills; each pill representing 0.25 gramme dried ox bile. To render these less liable to change in the stomach they were coated with salol—4 grammes to the 100 pills. Of these the patient took 30 daily, a total of 120 in four days, or 30 grammes ox bile and about 5 grammes salol.

Three months after the conclusion of this experiment the patient was operated upon again by Dr. Mixter. The common duct was found to be occluded with gall-stones of soft consistency. These were removed through an incision in the duct, and its complete patency into the duodenum once more established. The fistula ceased to discharge and the patient recovered. The patient then underwent another metabolism experiment of three days' duration, for the determination of her digestion under normal conditions of bile-secretion.

The following tables give the results of these two experiments and require no explanation.

Table I gives the ingesta of the patient during the first period, before bile was given.

Table II gives the ingesta of the patient during the second period, during which bile was taken by the mouth.

Table III gives the ingesta again without bile medication.

Table IV gives the total quantity of bile and bile-solids collected during the three different periods.

Table V gives the excreta of the three periods.

Table VI gives the ingesta in the experiment after the operation by means of which free communication through the bile-duct between the liver and duodenum was once more established.

Table VII gives the excreta in the experiment after the operation.

TABLE I.—INGESTA. 1ST PERIOD. BEFORE BILE MEDICATION.

Wgt. 65.2 kilog. Dec. 20–23, 1898.

Substance.	Amount.	Nitrogen.	Fat.	Carbohydrates.
Bread	592	8.0	2.7	335
Milk	2930	16.2	295.9	143
Sugar	290			290
Oatmeal	24	0.5		16
Tapioca	26			22
Eggs	346	7.0	42.0	
Ham	50	2.3	4.6	
Butter	158		136.7	
Cod Fish	53	2.8	0.8	
Roast Beef	94	3.1	8.9	
Apple Sauce	654	0.3		157
		40.2	491.6	963

ANALYSES OF INGESTA TAKEN DURING FIRST PERIOD.

Bread ..... 1.5229 g. contained 0.0207 g. Nitrogen or 1.36 %
1.9333 g. " 0.0260 g. " " 1.35 Average 1.355%
Fat reckoned at 0.46 %. Carbohydrates reckoned at 56.58 %

Milk ..... 2930 cc. in four days.

		Fat.		Nitrogen.	
Dec. 20....	910 cc.	11.144 %	11.084 %	0.5264 %	
Dec. 21....	490 cc.	8.340		0.5572	
Dec. 22....	800 cc.	11.000	10.854	0.5656	0.5796
Dec. 23....	740 cc.	9.104	9.116	0.5572	0.5572

Milk Sugar was reckoned at 4.88 %

Oat Meal ..... 2.4686 g. contained 0.0504 g. Nitrogen or 2.04 %
2.3086 " 0.0466 " " 2.01 Av. 2.025 %
Carbohydrates reckoned at 64.73 %. König's Tables.

Tapioca ..... Nitrogen neglected. Carbohydrates reckoned at 84.83 %, König.
3.8813 g. contained 0.0014 g. Nitrogen or 0.036 %
4.1760 " 0.0015 " " 0.037 Av. 0.365 %

Eggs ..... Nitrogen reckoned at 2.01 %. König.
Fat " " 12.11 "

Ham ..... 14.672 g. contained 1.3434 g. Fat or 9.15 %
Nitrogen reckoned at 4.62 %. See 2d Period.

Butter..... 3.6446 g. contained 3.1900 g. Fat or 87.53%
2.8756 " 2.4540 " 85.37 Av. 86.45 %

Cod Fish..... 6.8507 g. contained 0.1072 g. Fat or 1.56 %
12.2573 " 0.1750 " 1.43 Av. 1.49 %
2.4827 " 0.1305 Nitrogen or 5.25
2.6775 " 0.1394 " " 5.20 Av. 5.23 %

Roast Beef..... 38.0 g. contained 3.5796 g. Fat or 9.4 %
Nitrogen reckoned at 3.35 %

Apple Sauce.... 654 g. made up of 83 g. sugar and 571 g. apples. Carbohydrates in latter reckoned at 13.03 %. König. Nitrogen 0.058 %. König.

In addition to the above articles of food, one orange and a small amount of lettuce or celery and a cup of tea and coffee were given daily; also about 75 cc. of sherry in each of the three periods.

TABLE II.—INGESTA. 2D PERIOD. DURING BILE MEDICATION. DEC. 24–27, 1898.

Substance.	Amount.	Nitrogen.	Fat.	Carbohydrates.
Bread.....	472	6.4	2.1	267
Milk.....	3575	19.3	355.8	174
Sugar	341			341
Oatmeal	68	1.4		44
Tapioca	31			26
Eggs	560	11.3	67.8	
Ham	39	2.6	1.6	
Butter.....	114		98.6	
Cod Fish	43	2.2	0.6	
Apple Sauce.....	404	0.2		97
Hamburg Steak...	180	6.0	10.4	
Bile	30	0.5		
		49.9	536.9	949

ANALYSES OF INGESTA TAKEN DURING 2D PERIOD.

For analyses of bread, oatmeal, tapioca, eggs, butter, cod fish, and apple sauce, see 1st Period.

			Fat.		Nitrogen.
Milk	Dec. 24	1090 cc.	10.020 %	9.922 %	
	Dec. 25	865	9.518	9.528	0.5488
	Dec. 26	790	11.166	11.216	0.5166
	Dec. 27	830	9.080	9.324	0.5320
		3575 cc.			

Milk Sugar reckoned at 4.88 %.

Ham ..... 13.323 g. contained 0.5334 g. Fat or 4.0 %
3.662 g. " 0.1693 g. Nitrogen or 4.62 %

Hamburg Steak. 43 g. taken for analysis; dried to 12.3934 g. for analysis. 5.0414 g. contained 1.0682 g. Fat or 21.69 % or 6.25 % of undried steak.
3.8780 contained 0.7096 g. Fat or 18.30 % or 5.27 % of undried steak.
Average % of Fat = 5.76 %.

1.5856 contained 0.1847 g. or 11.65 % Nitrogen or 3.36 % of undried steak.

Bile ..... .009 g. Nitrogen in 2 pills. In 120 or 30 g., 0.54 g.

TABLE III.—INGESTA. 3D PERIOD. AFTER BILE MEDICATION. Dec. 28–31.

Substance.	Amount.	Nitrogen.	Fat.	Carbohydrates.
Bread	346	4.7	1.6	196
Milk	2240	11.4	224.9	109
Sugar	463			463
Oatmeal	27	0.5		17
Eggs	504	10.1	61.0	
Butter.....	96		83.0	
Hamburg Steak....	81	2.7	4.7	
Steak.....	254	9.0	25.1	
		38.4	400.3	785

ANALYSES OF INGESTA TAKEN DURING 3D PERIOD.

For analyses of bread, oatmeal, tapioca, eggs, butter and Hamburg steak, see 1st and 2d Periods.

Milk.	Amount.	Fat.		Nitrogen.
Dec. 28....	460 cc.	9.064 %	8.960 %	0.5320 %
Dec. 29 ..	520	9.764	9.752	
Dec. 30....	630	11.496	11.536	0.5348
Dec. 31....	630	9.890	9.856	0.4564
	2240 cc.			

Steak.

Dec. 29.... 88 g. 26 g. contained 1.9621 g. Fat or 7.55 %
Dec. 30.... 80 g. 33 " 5.1658 " 15.65
Dec. 31.... 86 g. 3.6753 " 0.7792 " 21.20 equiv. to 6.37 % undried steak.
3.0106 " 0.7362 " 24.45 " "
7.34 % undried steak. Average 6.86 %
1.7232 contained 0.2015 Nitrogen or 11.69 %
equiv. to 3.51 % " "
1.4864 " 0.1756 " " 11.81 %
equiv. to 3.55 % " " Average 3.53 %

TABLE IV.—Excretion of Bile in the three Periods.

Period.	Cannula.	Extracted from Dressings.	Total.	% Solids.	Total Solids.
I. Before Bile Medication,	2250	0	2250	1.37	30.83
II. During Bile Medication,	1429	1079*	2508	1.70	42.64
III. After Bile Medication,	1173	751*	1924	1.42	27.32

*The dressings were extracted several times with water until all trace of bile was removed. The extracts were then filtered and the filtrates evaporated and dried to constant weight. In Period II the dried bile-solids thus obtained were 18.3819 g. But the per cent of solids of the bile obtained in this period by the cannula was 1.70%. . . 1.70 : 100 : : 18.3819 : x = equivalent amount of bile obtained by extraction, i. e., 1079 cc. In Period III the dried bile solids obtained from the dressings amounted to 10.6772 g.— equivalent to 751 cc.

TABLE V.—Excreta. 1st, 2d, and 3d Periods.

URINE.

		Amount.	Sp. Gr.	Urea.		Nitrogen.	
I. Before Bile Medication,	Dec. 21	510	1029	14.6		8.1	
	Dec. 22	400	1024	10.1		5.6	
	Dec. 23	460	1024	12.7		7.2	
	Dec. 24	630	1018	13.5	50.9	7.6	28.5
		2000					
II. During Bile Medication,	Dec. 25	1224	1009	14.6		7.3	
	Dec. 26	840	1016	18.2		9.4	
	Dec. 27	700	1017	13.7		7.7	
	Dec. 28	670	1022	14.9	61.4	9.3	33.7
		3434					
III. After Bile Medication,	Dec. 29	670	1021	15.2		10.1	
	Dec. 30	650	1018	17.2		9.5	
	Dec. 31	650	1018	17.2		9.5	
	Jan. 1	270	1020	7.2	56.8	3.8	32.9
		2240					

STOOLS.

	Amount.	Nitrogen.	Fat.
I. Before Bile Medication,	423	1.79–7.6%	73.3–310%
II. During Bile Medication,	298	1.19–3.5	71.8–214
III. After Bile Medication,	290	1.51–4.4	78.1–226

Analyses of the Excreta of the three Periods.

1st Period. Stools. 423 g. contained 310 g. Fat and 7.6 g. Nitrogen.

5.0970 g. contained 3.7034 g. Fat or 72.66%
3.3647 " 2.4884 " 73.96 Average 73.31%

2.5962 " 0.0456 Nitrogen or 1.76
3.2571 " 0.0588 " 1.81 " 1.79

2d Period.	Stools.	298 g. contained 214 g. Fat and 3.5 g. Nitrogen.				
	3.7352 g. contained	2.7154 g.	Fat or	72.69%		
	5.0952 "	3.6166 "		70.98	Average	71.84%
	3.4500 "	0.0396	Nitrogen or	1.15		
	3.1200 "	0.0382 "		1.23	"	1.19
3d Period.	Stools.	290 g. contained 226 g. Fat and 4.4 g. Nitrogen.				
	4.6836 g. contained	3.5994 g.	Fat or	76.85%		
	4.2176 "	3.3432 "		79.27	Average	78.06%
	2.3090 "	0.0344	Nitrogen or	1.49		
	2.4366 "	0.0372 "		1.53	"	1.51

SUMMARY OF THE FIRST EXPERIMENT.

	Fat		Nitrogen		Bile Solids.	Balance	
	Ingesta	Excreta fæces.	Ingesta	Excreta fæces.		Excreta urine and stool.	Nitrogen.
I. Before Bile Medication.	491.6	310 or 63.1%	39.9	7.6 or 19%	30.83	36.1	+ 3.9
II. During Bile Medication.	537.1	214 or 39.8	49.7	3.5 or 7	43.64	37.2	+ 12.6
III. After Bile Medication.	400.3	226 or 56.5	38.4	4.4 or 11.5	27.32	37.3	+ 1.1

The digestion of fat was respectively 23.3 per cent and 16.7 per cent better in the bile period than in the 1st and 3d periods, in which no bile was given.

The digestion of nitrogenous food was respectively 12 per cent and 4.5 per cent better in the bile period than in the other periods.

The bile-solids were increased in the bile period 38 per cent and 60 per cent respectively above the amounts in the 1st and 3d periods. The excess of bile-solids in this period was equivalent to nearly one-half the amount of bile given during this time.

TABLE VI.—INGESTA IN THE EXPERIMENT AFTER THE OPERATION.

Weight 65.1 kilog. May 20–24, 1899.

Substance.	Amount.	Nitrogen.	Fat.	Carbohydrates.
Bread	423	5.8	1.9	239
Milk	2470	12.1	194.6	120
Sugar	363			363
Oatmeal	58	1.2		38
Tapioca	18			15
Eggs	707	14.2	85.6	
Butter	73		61.7	
Beef Steak	147	4.9	16.9	
Chicken	188	10.7	8.6	
Potato	229	0.5		44
		49.4	369.3	819

ANALYSES OF INGESTA TAKEN DURING THE EXPERIMENT AFTER THE OPERATION.

For bread, oatmeal, tapioca, and eggs, see analyses 1st Period. Experiment 1.

		FAT.		NITROGEN.
Milk				
May 2....	810 cc.	8.25 %	8.38 %	0.4662 %
May 3....	900	7.43	7.35	0.5166
May 4....	760	7.96	8.03	0.4914

Butter..... 2.3100 g. contained 1.9524 g. Fat or 84.5 %
1.9780 " 1.6714 " 84.4 Average 84.45

Beef Steak..... 5.6178 g. " 0.6450 g. " 11.48

Nitrogen reckoned at 3.36%—amount obtained at a subsequent analysis.

Chicken ..... 3.2096 g. contained 0.1616 g. Fat or 5.03 %
1.8238 " 0.1050 " 5.75 Average 5.39 %
3.1382 " 0.1770 Nitrogen or 5.63
2.9700 " 0.1711 " 5.75 " 5.69

84 g. of first portion of chicken given.

Chicken ..... 2d Portion 104 g. Nitrogen reckoned at 5.69 % as in 1st Portion.
2.4930 g. contained 0.0864 g. Fat or 3.46 %
3.8758 " 0.1386 " 3.57 Av. 3.52 %

Potato .....37.8 g. " 0.2630 g. Carbohydrates or 19.0 %
37.6 " 0.2616 " " 19.1 " 19·05
8.0 " 0.0172 Nitrogen or 0.215
8.0 " 0.0168 " " 0.21 " 0.21

TABLE VII.—EXCRETA DURING THE EXPERIMENT AFTER THE OPERATION.

URINE.

	Amount.	Sp. Gr.	Urea.	Nitrogen.
May 3.....	400	1032	8.8	5.2
May 4.....	1070	1017	23.2	11.8
May 5.....	975	1017	19.4	8.4
	2445		51.4	25.4

STOOLS.

Amount.	Nitrogen.	Fat.
145	5.9 g. or 4.1 %	54.8 g. or 37.8 %

ANALYSES OF EXCRETA DURING THE EXPERIMENT AFTER THE OPERATION.

STOOLS.

145 g. contained 54.8 g. Fat and 5.9 g. Nitrogen.

4.9832 " 1.9020 g. Fat or 38.1 % Average
5.8335 " 2.1826 " 37.4 37.8 %

1.4572 " 0.0596 Nitrogen or 4.1 Average
2.3256 " 0.0952 " 4.1 4.1 %

SUMMARY OF THE EXPERIMENT AFTER THE OPERATION.

FAT.		NITROGEN.	
Ingesta.	Excreta.	Ingesta.	Excreta.
369.3 g.	54.8 g. or 14.8 %	49.4 g.	5.9 g. or 11.9 %

SUMMARY OF THE TWO EXPERIMENTS.

	Per cent. of ingested fat lost in stools.	Per cent. of ingested nitrogen lost in stools.	Bile solids.	Urine per day.
I. Before Bile Medication,	63.1%	19.0%	30.83 g.	500 cc.
II. During Bile Medication,	39.8	7.0	43.64 g.	859 cc.
III. After Bile Medication,	56.5	11.5	27.32 g.	560 cc.
After operation,	14.8	11.9		815 cc.

Four other experiments were also performed—one on a human being and three on dogs with biliary fistulæ. Since these failed to be satisfactory in all particulars, only a brief resumé of this part of the work will be given.

Experiment 1 was performed upon a healthy woman, October 4-16, 1897. She was given in the second period of four days 20 grammes of dried ox bile. Notwithstanding the fact that the amount of fat in the food reached nearly 200 grammes a day, it was so well assimilated that it left no appreciable chance for improvement in its absorption during the bile period.

Experiment 2 was performed on a dog February 5-25, 1898. The common duct was ligated in two places and severed. The gall-bladder was then opened and a glass cannula, extending for 4 cm. beyond the abdominal wall, inserted. The dog recovered quickly from the operation and at the end of two weeks the wound was so well healed that he was ready for use. The diet consisted of dog-bread ground up and mixed with lard. The animal was kept in a cage with double bottom and fed twice a day. The experiment was divided into four periods, in the third of which the dog was given 12 grammes ox bile.

PER CENT. IN STOOLS OF INGESTA.

			Fat.	Nitrogen.
Feb. 5-10	I	Without muzzle No Bile	51.82 per cent.	24.31 per cent.
Feb. 10-15	II	With muzzle No Bile	45.87 per cent.	19.70 per cent.
Feb. 15-20	III	With muzzle Bile	59.95 per cent.	34.23 per cent.
Feb. 20-25	IV	With muzzle No Bile	33.15 per cent.	16.67 per cent.

The second period showed a loss of fat in the stools of 46 per cent as contrasted with 52 per cent in the first period. Such figures, however, are not conclusive when one is dealing with large amounts of fat.

The third and fourth periods are not of value because the dog had diarrhœa, due either to the bile pills or to the fat in the food. This not only interfered with the absorption of the fat, but prevented accurate separation of the stools.

Experiment 3. The dog was prepared in the same way as in the preceding experiments. He wore a muzzle throughout the experiment and for the three and one-half days preceding. The experiment was of 15 days' duration (June 30 to July 15, 1898), and divided into periods of five days each. In the second of these, the dog received 17.5 grammes of the dried ox bile.

Yet though the amount of fat in the diet was lessened from 80 grammes to 40 grammes a day, diarrhœa appeared as before aggravatingly near the end of the bile period. The results were, therefore, inconclusive.

PER CENT. IN STOOLS OF INGESTA.

	Fat.	Nitrogen.
I	41.9 per cent.	14.9 per cent.
II	45.3 "	12.4 "
III	63.0 "	22.1 "

Experiment 4. Dog. Common duct ligated and biliary fistula made June 18, 1898. In this instance the three periods were of two days' duration. In the middle period the dog took 10 grammes dried ox bile.

PER CENT. IN STOOLS OF INGESTA.

	Fat.	Nitrogen.
July 19-21,	46.1 per cent.	16.8 per cent.
July 21-23,	34.2 "	11.8 "
July 23-25,	74.5 "	31.8 "

But here again diarrhœa occurred toward the close of the experiment, vitiating the results.

The literature on this subject up to 1897 was given in detail by Pfaff and Balch [4] in their paper. Albu [5] has since brought the matter up to date. It will be only necessary therefore for me to state my conclusions.

[4] Op. cit.

[5] Zur Physiologie und Pathologie der Gallensecretion, *Berl. klin. Wochenschr.*, 1900, p. 866.

CONCLUSIONS.

1. Bile increases the digestion of fat when given by the mouth in pill form. The percentage of fat lost in the stools of our patient with a complete biliary fistula was 63 per cent in the first period and 57 per cent in the third. This closely corresponds to the results that Müller obtained in human beings and dogs with complete obstruction of the common duct. Under bile medication the stools contained 23 per cent less fat than in the first period, and 17 per cent less than in the third. This represents an actual diminution of the amount of fat lost in the stools. Looking at the result in another way, it may be said that the average digestion of fat in the periods without bile was 40 per cent; in the periods with bile, 60 per cent, *i. e.*, bile increased the digestion of fat relatively by 50 per cent.

2. The digestion of nitrogenous food is improved by the use of bile pills when the amount of fat in the stools is large. Instead of an average of 15 per cent being lost in the fæces, but 7 per cent escaped digestion during the four days the patient took bile. The reason for this, perhaps, lies in the better digestion of fat at this time, in consequence of which the proteid elements of the food were more thoroughly exposed to the digestive juices.

3. Ox bile is a cholagogue. The amount of bile-solids secreted in the bile period was 47 per cent greater than in the periods before and after. This confirms the work of Pfaff and Balch, here in Boston, on a human being, and that of Stadelmann[6] and his pupils, in Germany, on dogs.

4. The effect of the bile on the bowels in this case was not remarkable, although they moved more satisfactorily during the bile period. In my experiments with dogs I usually obtained diarrhœa when giving bile. I do not feel sure, however, that this should be attributed wholly to the medication, for the diarrhœas as a rule appeared six or more days after the beginning of the experiment and the animals were then in poor condition. Dr. Pfaff, who has had more experience with the administration of bile than I, tells me that he has found its action variable in patients. In some cases it is a laxative; in others, in

[6] *Zeitschr. f. Biol.*, 1897, n. F., xvi, p. 1.

which there is diarrhœa, due apparently to large amounts of fat in the food, it has the opposite effect.

5. As to the general effect of bile on body metabolism, it was observed that the urea and nitrogen were excreted in greater amount in the bile period than in either of the others. No definite conclusions can be drawn from this fact, because more nitrogen was ingested during these four days; moreover, it must be borne in mind that in these results the salol may have been a factor.

6. The amount of urine was increased by more than 50 per cent in the bile period. It is interesting to note that the amount was about the same during this bile period as in the second experiment when the bile was again taking its natural course. Von Noorden[7] has recorded a similar increase in the amount of urine following the removal of the obstruction in acute catarrhal jaundice. The salol coating of the bile pills, which amounted to one and a quarter grammes a day, is not sufficient to account for this effect. This is evident from the work of Kumagawa,[8] who gave two grammes of sodium salicylate daily to a dog of 25 kilos without essentially changing the amount of urine secreted. On the other hand, in taking the 30 pills daily the patient drank several extra glasses of water, and in the second experiment her general condition was naturally better than at any other time.

[7] Pathologie des Stoffwechsels, p. 281, Berlin, 1893.
[8] Virchow's *Archiv*, 1888, cxiii, p. 134.

THE RELATION OF DIABETES MELLITUS TO LESIONS OF THE PANCREAS. HYALINE DEGENERATION OF THE ISLANDS OF LANGERHANS.

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PLATE XXXIII.

Embedded in the substance of the pancreas are the peculiar bodies described by P. Langerhans and usually designated islands of Langerhans. They are composed of polygonal cells arranged in irregular columns, between which are wide tortuous anastomosing capillaries. The cells are of epithelial type and have the same origin as those of the ducts and secreting acini with which, at an early period of development, the cell columns are in continuity. The lumen of the ducts does not penetrate among the cells of the island, which is, therefore, not concerned in the elaboration of the pancreatic juice. These bodies resemble in architecture other ductless structures, such as the parathyroid bodies, the carotid and coccygeal glands, and less closely the suprarenal capsules, the pituitary body and the thyroid gland.

The intimate relation of columns of epithelial cells to a rich capillary network has suggested that they furnish some substance to the blood—the hypothetical internal secretion of the pancreas. Abundant experimental research, inaugurated by von Mering and Minkowski, having shown that the pancreas exerts some very important influence on carbohydrate metabolism, several writers—Laguesse, Schäfer, Diamare—have suggested that the islands of Langerhans perform this function. The only experimental evidence in support of this suggestion is furnished by Ssobolew, who, in a brief preliminary communication, states that after feeding animals with carbohydrates in considerable quantity the cells of the islands become

more granular than usual. He finds that the chronic interstitial pancreatitis, which in dogs follows ligation of the pancreatic ducts, spares the islands of Langerhans; this fact, he thinks, explains the absence of glycosuria.

In the preceding number of this JOURNAL[1] I have described alterations undergone by the islands of Langerhans in various forms of chronic interstitial pancreatitis, and have discussed the relation of these lesions to the disease of carbohydrate metabolism, diabetes mellitus.

Two forms of chronic inflammation of the gland are distinguishable: (1) interlobular pancreatitis, characterized by proliferation of fibrous tissue between the lobules, which are invaded from the periphery, and (2) interacinar pancreatitis, where the new-formed fibrous tissue is more diffusely distributed within the lobules and between individual acini. With the first type the islands of Langerhans are implicated only when the sclerotic process has reached a very advanced grade. To this variety belongs the chronic inflammation which follows occlusion of the pancreatic duct; though the secreting tissue of the gland is in very great part destroyed and replaced by dense fibrous tissue, the interacinar islands are not affected and persist for a long time as isolated cellular structures almost completely unchanged, though surrounded by scar-like tissue. They suffer only when the process is far advanced. With the interacinar type of inflammation, on the other hand, the islands are affected as are the other elements of the gland, and coarse strands of fibrous stroma following the capillary vessels separate the columns of atrophied cells.

Of eleven instances of chronic pancreatitis of the interlobular type, in only one was diabetes present. Here the inflammation which followed obstruction of the pancreatic duct had reached a very advanced

[1] Opie. On the relation of chronic interstitial pancreatitis to the islands of Langerhans and to diabetes mellitus. *Journal of Experimental Medicine*, 1901, v, p. 397. References to the authors cited are here given. See also *Journal of the Boston Society of the Medical Sciences*, June, 1900, iv, p. 251. W. Schulze (*Arch. f. mikr. Anat.*, August 31, 1900, lvi, p. 491) has recently shown that the islands of Langerhans are not implicated in the chronic interstitial inflammation which follows obstruction of the pancreatic duct in guinea pigs.

grade and the islands of Langerhans, isolated in the dense stroma, had undergone alterations. The accompanying glycosuria had been of only slight severity and had disappeared when the patient was upon a diet poor in carbohydrates. In two of three instances of interacinar pancreatitis diabetes mellitus was present. In the third case the lesion was slight and the organ weighed 170 grammes. Though the number of cases is small they indicate that where diabetes accompanies a lesion of the pancreas the islands of Langerhans are implicated in the disease.

In the same report I described a case of diabetes mellitus in which the pancreas was the seat of a very remarkable lesion. In sections from all parts of the organ were small areas in which the parenchymatous cells were replaced by hyaline material located immediately outside the walls of the capillaries. These areas, which were most abundant in the tail of the gland, frequently corresponded in size to islands of Langerhans, but were often much larger. Self-digestion of the gland in many places prevented the satisfactory study of the lesion, but even where the tissue was well preserved islands of Langerhans were not recognizable. Although the change began apparently in these structures, it had extended beyond their limits.

I have recently had the opportunity of studying the specimens from a case of diabetes in which the causal relation of a lesion of the islands of Langerhans to the disease is more clearly demonstrable than in those previously described. These bodies are the seat of a degenerative change which has left unaltered the secreting parenchyma of the gland.

Synopsis of Clinical History.—Female, negro, aged 54 years. The patient was admitted to the Johns Hopkins Hospital in the service of Dr. Osler, complaining of cough. Her family and personal history are unimportant. Her present illness began about eleven months before her admission, when, she states, she had a severe cold which became steadily worse. The cough has been accompanied by profuse expectoration. She has lost much weight. Several months after the onset of cough her urine increased greatly in quantity, so that for a time she was compelled to get up almost every hour during the night to void it. The urine was pale in color. At this time she experienced great hunger

and thirst and ate and drank enormously. These symptoms lasted during part of the spring and summer and disappeared some months before her admission to the hospital. She had recently voided the usual amount of urine and there was no excessive hunger or thirst.

When admitted the patient was thin but moderately well nourished. The mucous membranes were pale. The percussion note over the front of the chest and in the axillary region was hyperresonant; over the upper part of the back on the right side the note was dull, while elsewhere over the back there was flat tympany. The breath-sounds over the first and second interspaces on the right side in front were intensely tubular, while on the left side above the fourth interspace they had an amphoric quality and were almost cavernous. There was modified tubular breathing over the left back, amphoric over the upper part of the scapula. Numerous fine and coarse moist râles were heard throughout both lungs. A friction rub was audible in the lower left axilla. The sputum was abundant, yellowish green, muco-purulent in character, and contained numerous tubercle bacilli. The hæmoglobin was 62 per cent. The stools were of normal color and contained no fat.

The patient gradually became weaker. Cough was almost constant and large quantities of muco-purulent material were expectorated. The temperature was irregular, and until the day preceding death ranged between 98.8° and 102° F. Death occurred on the seventh day after admission and was not preceded by a period of coma.

The specific gravity of the urine varied between 1025 and 1035. It contained sugar in abundance; neither albumin nor casts were found. On the fourth day after admission 880 cc. were collected, the specific gravity was 1028 and 4 per cent of sugar was present. On the following day the amount was 1200 cc., the specific gravity 1035 and the quantity of sugar 5.4 per cent.

Autopsy.—Performed 51 hours after death. The body is that of a sparely nourished woman; the arms and legs are very thin and the abdomen is retracted. Subcutaneous fat is present in small amount.

The heart-muscle is pale, and upon the intima of the mitral valve are opaque, yellow patches of fatty degeneration. Within the coronary arteries near their orifices are a few slightly raised yellow sclerotic patches.

The left lung is very voluminous and is almost universally bound by fibrous adhesions to the chest wall. Immediately below the pleura, occupying the upper part of the upper lobe, is a large, irregular cavity, the walls of which are covered by yellowish necrotic material. The re-

mainder of the lobe is consolidated, dull yellowish red, mottled with areas of caseation and riddled with small cavities. The upper part of the lower lobe is very thickly studded with groups of confluent partly caseous tubercles. The right lung is also voluminous and bound at the apex to the chest wall by fibrous adhesions. Upon the pleura are sparsely scattered gray tubercles. At the apex below the pleura is a cavity, which in size and appearance resembles that of the left lung. The remainder of the lobe is in great part consolidated by numerous caseous tubercles; the tissue is tough in consistence and contains much fibrous tissue. Caseous tubercles are abundant in the middle and lower lobes. Upon the mucosa of the larynx near the posterior extremities of the vocal cords and upon the surface of the epiglottis are a few very superficial ulcers, the largest about 3 mm. in diameter.

The liver is pale and its lobulation is marked by gray-yellow and red mottling. Upon the cut surface are seen rather conspicuous yellowish points of minute size. The spleen is not enlarged.

The kidneys are of large size and weigh together 400 grammes. The surface, after removal of the capsule, is smooth and pale. The cortex, which has an average thickness of 5.5 mm., is of a grayish-red color. The left kidney has two separate pelves, from which arise two ureters opening by separate orifices into the bladder.

The mucous membrane of the stomach is normal in appearance. In the lower part of the ileum are a few scattered superficial ulcers about 0.5 cm. in diameter with irregular, slightly raised edges. On the peritoneal surface opposite one of them are several gray nodules which are just visible. A few similar ulcers are present in the large intestine.

The pancreas weighs 80 grammes and measures 23 x 5 x 1 cm. It can be readily dissected from the surrounding tissues. It is soft in consistence, and on section has a gray-yellow color.

The intima of the aorta, though fairly smooth, is studded with irregular, slightly raised plaques. The arteries at the base of the brain are normal in appearance. No lesion of the brain is found; the floor of the fourth ventricle presents nothing unusual. In the lower part of the right lateral lobe of the thyroid gland is a round firm nodule about 1 cm. in diameter. Its cut surface has a bright yellow color and is mottled with red and translucent gray.

Microscopic Examination.—In sections of the pancreas prepared for histological study is found no generalized increase of the interstitial tissue, but here and there, particularly in the tail of the organ, the

fibrous stroma shows some proliferation, and there are occasional irregular strands of tissue between the acini, though these are almost universally separated by delicate septa. This scanty, new-formed interstitial tissue, where it occurs, is poor in cells; about some of the medium-sized interlobular blood-vessels are small accumulations of scattered lymphoid cells, together with an occasional plasma cell. Mononuclear cells with eosinophile granulations are not infrequently seen in the interlobular and interacinar tissue. In the head and the body of the organ are small areas of post-mortem self-digestion, where nuclei no longer stain and the tissue takes with hæmatoxylin a diffuse blue tint. The glandular tissue elsewhere is well preserved. In many acini, centro-acinar cells are numerous. The ducts are not dilated and appear to be normal. There are no alterations of the veins or arteries.

The islands of Langerhans are the seat of a very remarkable change (Plate XXXIII). In varying amount within almost every island is a homogeneous material which stains with eosin. Only rarely is found an unaltered island. Those which are least changed contain a few scattered masses of hyaline material, of which the smallest are irregularly polygonal in shape and correspond in size to the cells of the island. The larger particles are rounded. This hyaline substance at times lies in the midst of groups of cells, but is usually in contact with the walls of the capillaries penetrating the island, or next the peripheral fibrous tissue, and is therefore usually between the remaining cells and the capillary walls. Increasing in amount, it replaces the cells and, where it is abundant, the cells which still persist are small and contain small nuclei, staining deeply with hæmatoxylin. They do not appear compressed or distorted.

Where hyaline material is abundant it forms conspicuous masses in contact with capillaries, the endothelium of which is well preserved. It does not form a uniform zone about them, but it occurs as scattered groups of irregular, rounded, often globular masses (Plate XXXIII, Fig. 2). The cells of the island have been in large part replaced, and between the hyaline particles is seen only an occasional compressed, fusiform or irregular nucleus.

The hyaline substance may occupy almost the entire area of the island, and besides a few endothelial cells are found only small scattered groups or rows of atrophic epithelial cells. The island is represented by a sharply circumscribed, hyaline structure, composed of particles of homogeneous material, giving the impression of broken, twisted columns, between which are the capillary walls. The nuclei of the capillary endothelium persist after destruction of the epithelial cells, but finally disappear. The lumen of the capillary remains patent and red blood-corpuscles are seen between the hyaline masses, although the endothelium no longer contains nuclei. The hyaline metamorphosis is limited strictly to the islands of Langerhans, the glandular acini remaining intact.

Lesions similar to those of the pancreas are not present in other organs. In the heart-wall are a few small areas where the interstitial tissue is increased, and in places the muscle-cells are fragmented, but other changes are not found. Sections from the lung show the histological pictures of conglomerate tubercles and of gelatinous and caseous pneumonia. The blood-vessels of the liver, spleen and kidney are apparently unaltered and there is no formation of hyaline material in these organs. Miliary tubercles are present in the liver, whose cells contain much fat.

The nodule observed at autopsy in the thyroid gland consists of altered parenchyma circumscribed by a thin circular capsule of fibrous tissue. The alveoli composing it vary greatly in size, some being very small and containing no colloid material, while others are large and irregular and distended with this material. The interstitial tissue of the nodule has an almost uniform hyaline appearance, when stained with eosin, and contains only very few scattered nuclei. The epithelium of the dilated alveoli is often much flattened. In places it is broken and the colloid within is continuous with the homogeneous substance replacing the interstitial tissue. Treated with Van Gieson's stain, the contents of the greater number of alveoli stain only with picric acid and assume a bright yellow color; but occasionally this material is colored by fuchsin and becomes deep orange red. The hyaline material immediately outside the alveoli and continuous, where the epithelial lining is broken, with that within, takes the same yellow stain and is doubtless extravasated colloid. Where it infiltrates the interstitial tissue midway between adjacent alveoli, it contains those constituents of the

fibrous stroma which have an affinity for fuchsin and, therefore, assumes a reddish color. The thyroid alveoli outside the nodule are far less irregular in size, and the stroma does not show the same uniform hyaline transformation, but here and there extravasation of colloid material has occurred into the interstitial tissue.

The peculiar transformation affecting the islands of Langerhans in this case belongs to the varied and ill-defined group of degenerative processes whose common characteristic is the formation of a homogeneous or hyaline material which stains with acid dyes, such as eosin and picric acid, but does not give the reactions of amyloid substance, though it resists the action of a variety of chemical substances, as strong acids and alkalies. These characteristics have been used by von Recklinghausen [2] to group together products of cell degeneration occurring in widely different tissues and doubtless representing a variety of essentially different processes, which even yet have received no satisfactory classification.

Under the heading of colloid transformation von Recklinghausen has included amyloid, hyaline and mucous degenerations—processes whose common character is the formation of a substance insoluble in the tissue juices. He recognized that the chemical and physical peculiarities ascribed to hyaline material do not serve to identify it as a true chemical compound, but he has grouped together as the products of related processes the hyaline substances which are formed in many different situations. These include the hyaline formed in the choroid of the eye, in certain tumors of the connective tissues and of the lymphatic apparatus; the homogeneous substances which are formed by certain glandular cells as products of secretion or of degeneration, namely, the contents of thyroid alveoli, hyaline renal casts, etc.; material formed on the surface and in the superficial layer of mucous membranes as the chief constituent of diphtheritic membranes; the so-called waxy or pseudo-waxy material formed by acute degeneration of smooth and striated muscle-fibres; the hyaline thrombi formed in the blood-vessels and in the heart.

Klebs [3] has attempted to classify more precisely these heterogeneous

[2] Handbuch der allgemeinen Pathologie des Kreislaufs und der Ernährung, p. 404. Stuttgart, 1883.

[3] Die allgemeine Pathologie, Theil ii, p. 100. Jena, 1889.

processes and to define more clearly certain long-accepted terms which have been applied to them. He reserves the name "colloid" for those albuminous substances which resemble the colloid of the thyroid gland and, like it, are elaborated by secreting cells, though not necessarily preformed within them. As "hyaline" he designates the firm, refractive and homogeneous albuminous material which does not give the reactions of amyloid and is formed in connective tissue and in other derivatives of the mesoderm. The hyaline material formed in and outside the cardiovascular system from constituents of the blood, the hyaline thrombi and hyaline exudates he distinguishes from the tissue hyaline produced by transformation of connective tissue.

By means of staining reactions, P. Ernst[4] attempts to demonstrate that the various substances grouped together by von Recklinghausen as hyaline are not chemically identical. Hyaline material from different sources, when treated with Van Gieson's mixture, exhibits in its affinity for acid fuchsin and picric acid differences which he attributes to peculiarities of chemical composition. That which is derived from epithelial cells stains orange yellow with the mixture of the two dyes, being colored by the picric acid and only tinted by fuchsin. Examples of this form of hyaline are the colloid of the thyroid gland and many renal casts. The second variety is formed in conective tissue or from coagulated material derived from the blood and stains intensely with fuchsin. Such hyaline occurs in the interstitial tissue of the thyroid gland, in certain tumors, as the hyaline remnants of the corpora lutea of the ovary, and as hyaline glomeruli in the kidney.

Lubarsch[5] regards the staining reactions of Ernst as an uncertain means for the identification of different hyaline substances and points out discrepancies between the criteria proposed by Klebs and by Ernst. Hyaline thrombi, which, according to Ernst, should stain red are usually yellow and only rarely stain deeply with fuchsin. The renal casts, which stain by Weigert's method for the demonstration of fibrin, stain orange yellow, though they are, as Ernst himself thinks, hyaline fibrin and, therefore, of parablastic origin. The colloid contents of the thyroid alveoli (as in the nodule described above), occasionally stain deep red, although this substance is undoubtedly of epithelial origin. Although Lubarsch discards the classification of Ernst, he admits that the method employed indicates in many cases the origin of hyaline

[4] Virchow's *Archiv*, 1892, cxxx, p. 377.

[5] *Ergebnisse der allgemeinen Pathologie und pathologischen Anatomie. Herausgegeben von Lubarsch u. Ostertag*, 1895, ii, p. 200.

material. Its advantage, he believes, lies in the fact that by it we can recognize whether hyaline contains a constituent derived from connective tissue. Normal fibrous tissue is stained intensely red by acid fuchsin and the substances which determine its affinity for the dye are present in the degenerate tissue. Pure hyaline, whatever its origin, Lubarsch suggests, always stains in the same way, while differences in staining reaction are dependent upon the admixture of other substances.

Hyaline material formed by a variety of processes from different tissues and exudates doubtless differs in chemical composition. That which is produced by coagulative necrosis from the protoplasm of parenchymatous cells and by the so-called pseudo-waxy degeneration of muscle-fibres is not identical with the hyaline of thrombi and of hæmatogenous exudates. The hyaline transformation of connective tissue in various situations, in certain tumors, in the walls of capillaries, in altered renal glomeruli, represents a longer continued process and is more closely related to amyloid degeneration. Since we have at present no means of determining the nature of these substances, their source and their methods of production can alone be used as a basis of classification. Following Lubarsch, we may distinguish hyaline of epithelial and of conjunctival origin, and again we may recognize that, like the colloid of the thyroid gland, formed outside of cells presumably by a process of secretion, and that formed by transformation of the cell protoplasm.

The material which in the case herewith described partly or completely replaces the islands of Langerhans, was tested with a variety of agents which have been used in the study of hyaline substances. It stains deeply with acid dyes, like eosin and picric acid, but shows little affinity for nuclear stains, as, for example, hæmatoxylin and methylene blue. With basic fuchsin and water blue, by the method which Unna[6] has used for the demonstration of basiphilic hyaline, it is stained only by the acid dye. The tissue available for study after discovery of the lesion had been hardened in Zenker's fluid. The methods employed by Pianese[7] in studying the hyaline degeneration occurring in cancer cells require in great part special methods of fixation and were not used.

[6] *Monatshefte für prakt. Dermatol.*, 1894, xix, p. 663.

[7] Ziegler's *Beiträge z. path. Anat.*, Suppl. Heft, 1896, p. 684.

The reactions of amyloid were not obtained with iodine, nor with gentian violet, methyl violet nor iodine green. The material did not stain by Weigert's method for the demonstration of fibrin.

Of much interest, in view of the study of Ernst, is the behavior of the substance toward picric acid and acid fuchsin. It stains with picric acid, but shows no marked affinity for acid fuchsin. The result varies slightly when varying proportions of the two substances are used. With a mixture of 3 parts of concentrated aqueous solution of acid fuchsin and 150 parts of concentrated aqueous solution of picric acid (Van Gieson-Ernst) the hyaline is stained yellow tinted with red. Using the two stains, as employed by Unna, a pure yellow results; the minute strands of fibrous tissue which penetrate the island stain deep red, while the hyaline material in contact with them is yellow.

This material, therefore, conducts itself toward Van Gieson's stain as does, according to Ernst, hyaline of epithelial origin, and in its staining reaction resembles the colloid material which is present in the greater number of thyroid alveoli and which in the thyroid nodule described has found its way into the interstitial tissue. Accepting the interpretation of the reaction suggested by Lubarsch, it does not contain the constituent of fibrous tissue upon which depends an affinity for acid fuchsin. It may be here noted that in the thyroid nodule, where extravasated colloid has infiltrated the fibrous tissue between adjacent alveoli, it has assumed a reddish color.

The staining reactions of this homogeneous substance present in the islands of Langerhans, interpreted with reserve, indicate its epithelial origin. Finding it in irregular masses in contact with the cells, I was at first inclined to believe that it was formed by a process resembling secretion. It was pointed out to me by Dr. Welch, who examined my specimens, that transitions occurred between the cells and the hyaline masses. In slightly altered islands one finds small masses of material which resemble the hyaline substance, but, like the cell protoplasm, have a granular aspect. They correspond in size to the adjacent cells of the island but contain no nuclei.

By the use of certain stains, phosphomolybdic acid hæmatoxylin

by the method of Ribbert for white fibrous tissue or aniline blue, as employed by Mallory,[8] for the demonstration of white fibres and reticulum, the hyaline material acquires a deep blue color and becomes very conspicuous. In the islands are not infrequently found slightly enlarged cells which, though still containing nuclei, exhibit a reaction similar to that of the hyaline material. The cell protoplasm, though granular, has assumed a diffuse blue color.

The degenerative process first manifests itself by an increase in the size of the cell and an alteration of its protoplasm. With the death of the cell its nucleus disappears and the protoplasm which stains with acid dyes remains for a time granular, but subsequently becomes homogeneous. The small particles of hyaline fuse with one another and form larger masses which lie in contact with the fibrous septa of the island. After complete transformation of the cells the island is represented by a hyaline mass penetrated by the remains of altered capillaries.

In the preceding number of this JOURNAL[9] I described, with an illustrative drawing, a case of diabetes in which hyaline material was present in circumscribed areas throughout the pancreas. The staining reactions of this substance resembled those observed in the present case. Amyloid reactions were not obtained, nor did it stain by Weigert's method for the study of fibrin. With the various modifications of Van Gieson's mixture, it showed little affinity for acid fuchsin. With the methods of Ribbert and of Mallory previously mentioned, it assumed a deep blue color. The deposition of calcium salts within it gave evidence that the process was of long standing. Unlike that of the present case, this substance was deposited in fairly compact tortuous columns; the areas of hyaline transformation frequently corresponded in size to islands of Langerhans, but in many cases had evidently extended beyond their limits; transitions between the epithelial cells and the hyaline substance were not noted. Owing to the poor preservation of the tissue, it was not possible to study the early stages of the condition. It is nevertheless not improbable that the lesions in the two cases are of similar nature.

[8] *Journal of Experimental Medicine*, 1900, v, p. 15.

[9] Op. cit., p. 419.

Varied experiments performed upon the pancreas of animals have conclusively shown that this organ is essential to normal carbohydrate metabolism, and abundant clinical and pathological observation has demonstrated that the conclusions based upon these experiments are applicable to man. In my previous study I have briefly summarized the experimental and clinical evidence which establishes the causal relationship of certain destructive lesions of the pancreas to diabetes mellitus.

Impaired pancreatic function is doubtless not the only cause of diabetes, and in many instances of this disease no alteration of the organ has been demonstrable. On the other hand, diabetes is present in only a limited number of the cases where pancreatic disease has existed. It was the object of my preceding article to show that, where diabetes is caused by a lesion of the pancreas, the lesion is of such a character as to destroy or injure the islands of Langerhans, and that where, though the organ is diseased, diabetes is absent, the interacinar islands are relatively unaffected. In the cases (Nos. XIV and XV) of chronic interacinar pancreatitis, accompanied by diabetes, sclerosis of the organ was so slight that it was definitely recognized only by the microscope, while in instances (Nos. XI and XII) where the lesion followed obstruction of the pancreatic duct, diabetes was absent, though the organ was in great part converted into fibrous or fatty stroma. In the former cases the lesion affected the islands of Langerhans, while in the latter they were spared, though the surrounding secreting tissue was destroyed. In no instance were the islands destroyed, while the secreting structures remained intact.

Structures so embedded as are these islands in the substance of the gland cannot by any means now at our disposal be subjected to experimental alterations without injury to the surrounding alveoli. In the pancreas which has been described in this article, a lesion of obscure etiology has destroyed the cells of the islands of Langerhans, while those of the secreting acini, as well as those of other organs, are unaffected. The most successful experiment could not more accurately have selected these bodies. The association of diabetes mellitus with this lesion affords, I believe, convincing proof of the inferences drawn from the preceding series of cases.

Destruction of the pancreas in animals and in man is accompanied by diabetes; in the present case destruction of the islands of Langerhans has been accompanied by this disease. Since diabetes is absent when, as the result of duct obstruction, the secreting portion of the gland undergoes great alteration, though the islands are spared, the conclusion is justified that it is those structures which influence carbohydrate metabolism. What has been learned concerning the relation of the pancreas to diabetes is the relation of the islands of Langerhans to this disease.

DESCRIPTION OF PLATE XXXIII.

Fig. 1. Drawing made with low magnification (Leitz Oc. 3; Obj. 3) showing hyaline transformation of islands of Langerhans.

Fig. 2. Drawing made with a higher magnification (Leitz Oc. 3; Obj. 6) showing an island whose cells are partly transformed into hyaline material.

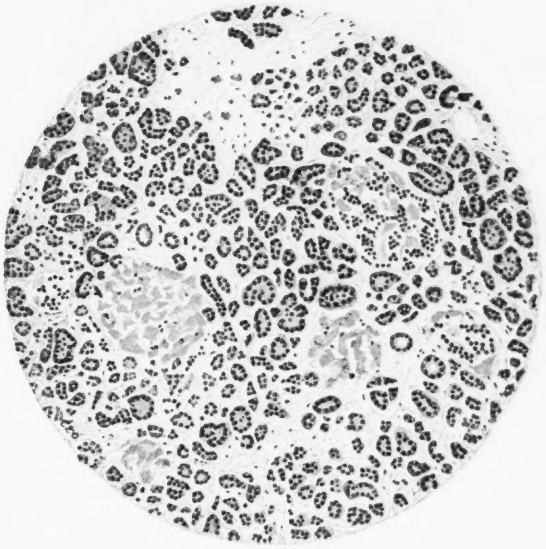

FIG. 1.

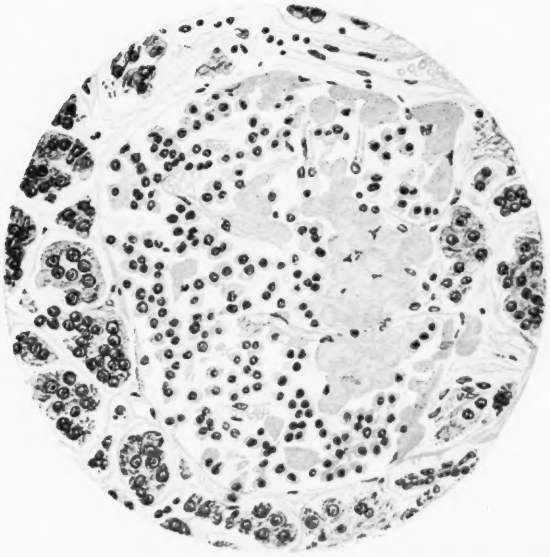

FIG. 2.